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5530

Some Observations on the Pharmacological Action of Gelsemicine, an Alkaloid from Gelsemium Sempervirens, L.

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Gelsemicine is a new alkaloid isolated by Chou[1] from the rhizome and roots of the American *Gelsemium sempervirens, L.* This alkaloid is the most toxic yet isolated. Using gelsemicine hydrochloride the minimum lethal dose for male rabbits given intravenously was found to be 0.05 mg. per kilo; for dogs 0.6 mg. per kilo; subcutaneously for albino rats 0.0001 mg. per gram, and subcutaneously for frogs 0.03 mg. per gram.

The toxic symptoms observed for the mammals were similar. There was a preliminary quietening of the animal with some slowing of the respiration. Tremors followed by incoordination of movement, and loss of power developed in 5 to 15 minutes. At the same time the respiration became either slow and deep or rapid and shallow, the result being a decrease in minute volume. In 15 to 30 minutes after the injection there were developed intermittent tetanic convulsions alternating with great prostration. Respiration now became slow and shallow. The animal usually gasped for breath, grunting and crying at times. Defecation and urination usually occurred. In the dog there were marked salivation, retching, and

[1] Chou, *Chinese J. Physiol.*, 1931, in press

sweating of the paws. Respiration rapidly became weaker until it finally stopped. Heart beats remained strong for 15 to 30 seconds after the cessation of respiration. Consciousness was apparently retained until a few minutes before death.

With a sublethal dose the animal remained quiet after the last convulsion and the respiration gradually returned to normal. There was complete recovery one to 2 hours after the injection.

Smaller doses produced only some quietening of the animal and some lessening of pain sensation. There was a very slight degree of narcosis.

Dogs under luminal or ether anesthesia were much more susceptible to gelsemicine hydrochloride. One-tenth of the ordinary minimal lethal dose caused death from respiratory failure in a few minutes.

Action Upon Respiration. Dogs under luminal anesthesia showed an increase in amplitude as well as in rate of respiration when a dose smaller than one-tenth of the toxic dose was given. With a larger dose there was depression preceded by a transient stimulation. Lethal doses produced no stimulation but a progressive depression until paralysis finally set in. Atropinization did not modify this action of gelsemicine. Normal rabbits exhibited the same findings. There was no antagonism but rather a synergism between a small stimulative dose of gelsemicine and a depressive dose of morphine.

Action Upon Circulation. Circulation was not affected until some time after a marked depression of the respiration. When asphyxia set in there was a slowing of the heart rate and a lowering of blood pressure. Peripheral organs such as leg, intestine and nose showed no change until there was a marked weakening of the respiration. The leg volume then decreased, the intestinal volume increased. The spleen showed an increase in volume with a dose which stimulated the respiration and a marked decrease with a dose which depressed respiration.

That there was no direct action upon the mammalian heart was shown by observation upon a dog with a heart oncometer. As long as the respiration was kept up there was no essential change of the tracing even with 20 times the minimal lethal dose for anesthetized animals.

Perfusion of the frog heart showed that gelsemicine HCl in a concentration of 1:1,000,000 produced a stimulation of the heart rate and amplitude. With a concentration of 1:500,000 there was no change or only a slight stimulation followed by moderate depression. When the concentration was 1:50,000 there was a progressive

slowing of the heart beat with a decrease in amplitude until finally the ventricular beats became irregular or even stopped.

Action on the Isolated Intestine and Uterus. Freshly isolated intestine and uterus from rabbits and dogs were used. The tissue was suspended in Locke's solution kept at 38°C. Gelsemicine HCl in a concentration of 1:1,000,000 in Locke's produced an increase in the amplitude as well as in the rate of contractions of the isolated intestine of rabbit or dog. Higher concentration resulted in an increase in tone followed by a diminution both in amplitude and rate. When the concentration of the gelsemicine HCl was increased to 1:100,000 the decrease in rate and amplitude was very marked and the tone was also decreased. In this stage the action of barium chloride was completely antagonized. Atropinization did not alter the action of gelsemicine.

Isolated rabbit or dog uterus reacted toward gelsemicine somewhat differently from the intestine in that there was a marked preliminary increase in tone with all the concentrations given above. There was a greater increase with the higher concentration, followed by a gradual decrease of tone until it was lower than normal. The normal rhythmical movements decreased in both rate and amplitude. The curve obtained simulated that with barium chloride although the action of the latter was partially antagonized. Ergotoxinization and atropinization did not alter the action of gelsemicine.

Action on the Pupil. Two rabbits were tried. After application of a drop of 0.2% solution of gelsemicine hydrochloride a gradual mydriasis developed in about 20 minutes. The mydriasis was not complete but the light reaction was eventually lost. Both animals died in about one hour after the administration of the drug, apparently due to rapid absorption from the conjunctiva.

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Acid-base Paths in Human Subjects.

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Using the micro-acid-base technique[1] a study has been made of

[1] Shock, N. W., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 780.

the acid-base variation under experimental conditions of 6 patients (2 cases of nephrosis, 2 of diabetes, and 2 of nutritional edema). The normal acid-base balance was displaced toward the acid side by the administration of ammonium chloride or hydrochloric acid, and toward the alkaline side by the administration of sodium bicarbonate. Frequent determinations of the acid-base balance were made during the course of the displacement from and the return to normal. Approximately 7 determinations were made during the 8 hours following the administration of the salts, and several determinations were made on the succeeding days.

The results when plotted on a triaxial coordinate chart may be described as follows: After a change is produced in the serum bicarbonate by fixed acids or alkalies the path taken by the blood is toward a changed pH and CO_2 tension. The change in pH is approximately 2 times the corresponding change in CO_2 tension when the latter is expressed logarithmically. The direction may be stated as being approximately at right angles to the direction taken by the CO_2 absorption curve of blood plotted on the triaxial coordinate chart.

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Coagulation of Egg Albumin by Supersonic Waves.

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Schmitt, Olson and Johnson[1] observed that solutions of egg albumin when exposed to supersonic waves became turbid and the albumin precipitated out in fine shreds. Not only is the mechanism of this novel method of coagulation entirely unknown, but it is not even certain whether the coagulation was caused by the mechanical vibration itself or by the heating effect of electric and supersonic waves, since these investigators did not take the precaution to cool the solution during exposure. The present communication extends their observation and examines the mechanism of coagulation by supersonic waves.

The oscillating current was generated by two 75 watt tubes (UX. 852) connected in a modified Hartley circuit. The quartz

[1] Schmitt, F. O., Olson, A. R., and Johnson, C. H., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 718.

plate (about 47x50 mm.) lay between 2 brass electrodes the distance between which could be adjusted. The vibrating part was placed in a dielectric liquid, usually benzene. The quartz plate vibrated at the rate of 1.3×10^6 times per second. On the upper electrode was placed a coil of glass tubing through which cold water circulated.

By adjusting the distance between the 2 electrodes it is easy to produce a mount of several centimeters above the surface of the liquid. We found that the effect of the vibration is greatest at the mount.

About 5 cc. of 1% egg albumin solution was placed in a test tube 15 mm. in diameter. The tube was held so that its bottom was just buried in the mount. A thermometer was placed in the tube. The pH of the solution was about 4.8.

Immediately upon exposure to the waves tiny particles of coagulated albumin are formed throughout the solution. These particles show rapid vibration, but they eventually rise to the surface. Under the microscope they are seen to consist of shreds enclosing air bubbles. The temperature of the solution did not rise more than 2 degrees above the room temperature, thus excluding the possibility of coagulation by heat.

The occlusion of air by the particles of coagulated albumin suggests that air bubbles might be the cause of the coagulation. Accordingly, we prepared some gas-free albumin solution by extraction in Van Slyke's apparatus and carefully transferred the solution to a specially constructed tube out of contact with air. The gas free albumin solution when exposed to the supersonic waves showed no sign of coagulation.

We prepared also gas free albumin solutions and resaturated them with H_2, O_2, CO_2 or H_2S. With H_2 and O_2, coagulation occurred when the solution was exposed to the waves, but no coagulation was observed in solutions saturated with CO_2 or H_2S. The absence of coagulation in CO_2 and H_2S solutions was not due to the acidity, because albumin solutions saturated with air and brought with acetic acid to the same acidity as that of the CO_2 solution showed coagulation.

To explain the difference between air H_2 and O_2 on the one hand and CO_2 and H_2S on the other, we exposed solutions of these gases (without albumin) to the supersonic waves. In solutions saturated with air, H_2 or C_2 gas bubbles were formed immediately upon exposure, while CO_2 and H_2S solutions gave off no gas. We are not certain whether this difference is due to the different solubilities of

the gases, but it is clear that gas bubbles are essential in the coagulation of albumin by supersonic waves. The rapidly vibrating gas bubbles on their ascent to the surface cause coagulation of the albumin at the interface much in the same way as albumin is coagulated by shaking which has been shown to be a surface phenomenon.[2]

5533

Stimulation of B. typhosus Agglutinin in Typhus Fever.

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It has long been recognized that the agglutinin for typhoid bacilli as shown by the Widal reaction is often encountered during typhus fever. In our experience here we observe that the sera obtained from proved cases of typhus may show early Widal test up to a higher titre while the Weil-Felix reaction or the presence of agglutinin for *B. proteus* X19 may appear later or in a much lower titre. Some of these cases may give the history either of previous typhoid infection or prophylactic vaccination, but in others none of these factors could be definitely ascertained. It has been suggested by some observers that during typhus fever the appearance of the Widal reaction is due to non-specific stimulation of latent or low titre antibodies by the fever.[1]

In a recent observation, however, it has been found that experimental typhus in animals (guinea pigs, rabbits and monkeys), previously vaccinated with typhoid bacilli, had no influence on the Widal reaction.[2] This is contradictory to actual findings made in human beings. In view of the value of *B. proteus* X19 as an antigen for the diagnosis of typhus fever, it seems to us of interest to determine whether the introduction of that organism into typhoid inoculated animals would stimulate the production of anti-typhoid agglutinin.

In our present experiment we are not concerned with the action of other microorganisms in this direction, since non-specific stimulation of specific antibodies has been recorded on experimental ani-

[2] Wu, H., and Ling, S. M., *Chinese J. Physiol.*, 1927, **1**, 407.

[1] Felix, A., *J. Hyg.*, 1929, **28**, 418.

[2] Reimann, H. A., and Wu, C. J., *J. Immunol.*, 1930, **18**, 159.

mals through the use of certain heterologous bacteria[3] that have no relation whatsoever with typhus fever.

For the purpose of our study, a series of 5 rabbits was inoculated with *B. typhosus*. Altogether 4 injections were given at weekly intervals, the first being 1/10 of a heat killed agar slant culture given subcutaneously, and the others in increasing doses of 1/40 to 1/10 of a living culture administered intravenously. Similarly, another series of 5 animals was immunized with *B. proteus* X19. Following this each animal of both series was bled at regular intervals from the 7th to the 45th day after the last injection and the agglutinin titres determined by standard macroscopic method. The agglutinin in titre for the typhoid series ranged from 1:10,240 to 1:20,480 the first week after the last injection and those for the proteus series were practically the same. No cross agglutination was present. Subsequent weekly examinations showed that after 45 days, the anti-typhoid and anti-proteus agglutinins have fallen to a titre of 1:640 or 1:280.

At this period another course of inoculations was given to serve as a non-specific stimulating agent. The animals which were immunized with *B. typhosus* received now injections of *B. proteus* X19 and *vice versa*. Altogether 4 injections were given to each animal at weekly intervals in increasing doses of 1/5,000 to 1/50 of a living agar slant culture. The animals were bled at regular intervals from the 7th to the 60th day after the last injection. It was found that the group of rabbits immunized with *B. typhosus* and subsequently stimulated by the injection of *B. proteus* X19 showed considerable increase in the agglutinin for typhoid bacilli rising from a titre of 1:1,280 to that of 1:81,920, whereas the agglutinin titre for *B. proteus* X19 was not stimulated as shown in the table. At the same time, the animals immunized with *B. proteus* X19 and then stimulated by the inoculation of *B. typhosus* also showed a higher agglutination titre for the latter, but for the former there was no appreciable increase. Control animals injected throughout with either *B. typhosus* or *B. proteus* X19 only showed no cross agglutination in their blood.

As summarized in the table it has been found that in rabbits, inoculation of *B. proteus* X19 results in a considerable stimulation of a low titre or latent anti-typhoid agglutinin, whereas the inoculation of *B. typhosus* fails to stimulate markedly a pre-existing low titre anti-proteus agglutinin. This finding is in agreement with the observations recorded in human cases of typhus fever and like the

[3] Dreyer, G., and Walker, E. W. A., *J. Path. and Bact.*, 1909, **14**, 28.

TABLE I.

Agglutination titres determined on various days after the last injection following immunization and subsequent stimulation.

Rabbit No.	Immunized with	Agglutination titre				Stimulated with	Titre after stimulation			
		B. typhosus		*B. proteus* X19			*B. typhosus*		*B. proteus* X19	
		7 days	45 days	7 days	45 days		7 days	60 days	7 days	60 days
1	*B. typhosus*	20,480	1,280	0	0	*B. proteus* X19	40,960	1,280	5,120	160
2		10,240	1,280	0	0		40,960	1,280	10,240	160
3		10,240	1,280	0	0		81,920	640	10,240	160
4		20,480	1,280	0	0		40,960	640	2,560	80
5		10,240	1,280	0	0		81,920	1,280	2,560	80
6	*B. proteus* X19	0	0	20,480	640	*B. typhosus*	81,920	640	1,280	80
7		0	0	10,240	1,280		20,480	640	640	80
8		0	0	20,480	1,280		40,960	1,280	2,560	160
9		0	0	10,240	640		40,960	1,280	1,280	160
10		0	0	20,480	640		20,480	640	1,280	80

Titre 0 = a negative result in dilution 1:20.

agglutinins for the Weil-Felix reaction in the blood serum of such patients, the agglutinin for *B. Proteus* X19 does not respond so readily to a non-specific stimulus.

5534

Resistance of the Eye to Bacterial Infection Following Neuroparalytic Keratitis.

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Neuroparalytic keratitis is regarded as a trophoneurosis caused by injury of the Gasserian ganglion or nerves emanating from it or of the ciliary ganglion. Recently we succeeded in the experimental production of neuroparalytic keratitis through the injection of one cc. of 80% alcohol into the rabbit's orbita.[1] Immediately after the retrobulbar injection of alcohol the cornea loses its sensitivity and in 24 hours the corneal epithelium begins to exfoliate. This usually is associated with more or less profuse discharge from the cul-de-sac and chemosis of the bulbar conjunctiva. Keratitis of various degrees develops in the course of 3-7 days after the injection. In some cases this process is aggravated by the ulceration of the cornea characteristic of neuroparalytic keratitis. Out of 34 experiments done in connection with this study, neuroparalytic keratitis failed to develop only in 4 instances.

Among the hypotheses on the pathogenesis of this disease, one supports the bacterial origin of the affection. Davies and Hall[2] found a pseudo-diphtheritic type of bacillus in human eyes affected with neuroparalytic keratitis to which they attributed the specific etiological rôle. Other authorities are of opinion that neuroparalytic keratitis is due to a secondary infection of the eye, the resistance of which has been diminished by the trophic disturbance of the nervous supply of the organ.

The readiness with which neuroparalytic keratitis can experimentally be produced in the rabbit's eye has offered means of investigating the question of the bacterial origin of this disease and the probable diminution of the resistance of the injured eye to bacterial infection. We at first determined the normal bacterial flora of the rabbit eyes using blood agar plates for culturing the content of the cul-de-sac. It was found that the cultures were constantly negative.

A series of rabbits were then treated with the retrobulbar injection of alcohol and the bacterial flora of the eyes was studied. Daily cultural examination of the material from the eyes has shown that

[1] Soudakoff, P. S., An experimental study on neuroparalytic keratitis, in press.

[2] Davies, H. M., and Hall, G., *Brit. Med. J.*, 1908, **1**, 71.

the development of neuroparalytic keratitis was regularly associated with the appearance of *Staphylococcus albus* or *flavus* or, sometimes *Micrococcus catarrhalis*. The pseudo-diphtheretic type of bacilli was never isolated. This finding suggested that the activity of these microorganisms may be responsible for the morbid process. To elucidate this point a group of 6 rabbits was treated with alcohol in the same manner after which the animals received daily instillation of 25% argyrol. The content of the cul-de-sac, taken every day before instillation of the drug, showed no growth, but nevertheless 5 rabbits in this group of animals developed neuroparalytic keratitis. In 2 cases which at first were culturally negative, staphylococci appeared in the later course of the disease. In these 2 instances, the condition was complicated by hypopyon and extensive ulceration of the cornea. From this observation it may be concluded that the development of the neuroparalytic keratitis in rabbits is independent of the activity of the bacteria spontaneously present in the injured eye. It cannot be excluded, however, that the ulceration of the cornea may be due to a secondary infection by pyogenic microorganisms. Though it is clear that the microorganisms found are not connected etiologically with the disease, their regular presence in the experimental eye may indicate that their development is due to the lowered resistance of the eye. If such a reasoning was correct, it would be logical to expect that other pathogenic organisms could be easily inoculated into the injured eye. To verify this point the following experiment has been conducted.

One cc. of 80% alcohol was injected into the right orbita of 5 series of rabbits. On the second day after the injection a 24-hour broth or blood agar culture of different microorganisms was gently rubbed into the conjunctiva and cornea of both eyes, the fellow eye being used as a control. To assure "intake" 3 inoculations were given at 2 or 3 days' interval. For the experiment we selected the microorganisms which might under certain conditions be pathogenic for human eye, namely, *Streptococcus hemolyticus*, Pneumococcus type III, *B. diphtheriae*, *B. pyocyaneus* and *B. subtilis*.

A group of 3 rabbits received repeated inoculations of *Streptococcus hemolyticus*. The cultural examination showed that no organism could be isolated from normal eye even 20 hours after the inoculation. The affected eye was usually positive for *Streptococcus hemolyticus* cultures only during the first 24-36 hours after each inoculation. It seems that *Streptococcus hemolyticus* remained viable in the injured eye for somewhat longer time than in a normal eye. This, however, has not modified the usual course of the neuro-

paralytic keratitis in rabbits. A second group of 3 rabbits was inoculated with pneumococcus type III. These animals also showed no apparent variation in the course of the neuroparalytic keratitis. Likewise positive cultures for pneumococcus were secured only 24-36 hours after inoculation, while from normal eye the microorganism was eliminated within few hours. A third group of 6 rabbits received the inoculations of *B. diphtheriae*. Two rabbits developed marked pseudo-membranes upon the conjunctiva of both eyelids. One of these rabbits died in a few days of apparently general toxemia, another animal recovered. In both cases the cultures gave rich growth to *B. diphtheriae*. In those instances in which no formation of pseudo-membranes was noticed, the cultures made within 24 hours after inoculation usually showed no growth of *B. diphtheriae*.

From the data presented above, it follows that the development of experimental neuroparalytic keratitis in rabbits following retrobulbar injection of alcohol is not determined by pathogenic bacterial activity. Though such injections result in a severe reaction manifested by various pathological processes in the eye tissue, no evidence has been found to support the hypothesis that trophoneurotic changes in the eye may alter the normal resistance of this organ.

5535

The Toxicity of Gelsemium.

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The rhizome and root of *Gelsemium sempervirens*, Linne, indigenous to North America, have long been used in medicine for their analgesic and antispasmodic properties and are actively poisonous. It is recorded that 0.8 cc. of the fluid extract proved fatal to a child of 3 years and many other cases of gelsemium poisoning are studied by Wormley,[1] Witthaus,[2] and others. Gelsemine, the principal alkaloid of gelsemium, was first investigated by Wormley,[3] Sonnenschein,[4] and Gerrard,[5] and by Moore,[6] who isolated it in its pure state. Thompson, in 1887,[7] obtained from the plant a second amorphous alkaloid, gelseminine, which was found by Cushny[8] to

[1] Wormley, *Am. J. Pharm.*, 1882, **54**, 337.

[2] Witthaus, *Medical Jurisprudence, Forensic Med. and Toxicology*, 1911, **4**, 937.

be highly poisonous, 1 mgm. of its hydrochloride being fatal to a rabbit weighing 2850 gm., while gelsemine was inactive to mammals, but produced strychnine-like effects in frogs. Sayre and colleagues[9] showed that gelseminine was not a single substance, but a mixture of 3 alkaloids, to which the names, sempervirine, gelsemidine and gelsemoidine were given; sempervirine being crystalline and the other 2 amorphous, but none of them were as potent as gelseminine itself. In reinvestigating this important drug of American origin, the writer[10] isolated 2 crystalline alkaloids, gelsemine and a new alkaloid, gelsemicine, which has a formula $C_{20}H_{25}O_4N_2$, a melting point 171°C. and a specific rotation —140°. Gelsemicine is highly potent and produces the usual toxic effects in mammals. A 0.1% solution of its hydrochloride dilates the pupil of rabbits for more than 4 hours. M.L.D. of gelsemicine hydrochloride was recently found by Hou[11] to be 0.05 mg. per kilo. A third crystalline alkaloid, to which the writer gives the name sempervine has now been isolated from the rhizome and root of gelsemium. It is very similar to Sayre's sempervirine in its general chemical behavior but differs from the latter in its melting point. It crystallizes out from chloroform in blood red prismatic needles, melting at 223°C., and from alcohol in red orthorhombic crystals melting at 254°C.; both of them, however, give rise to the formation of the same nitrate melting at 283°C. with decomposition. Sempervine is not so toxic as gelsemicine. Hou[11] found that 5 mgm. of sempervine hydrochloride per kilo body weight proved fatal to rabbits and 0.1 mgm. per gram body weight to frogs. It produced convulsion and other gelsemium poisoning effects in mammals, but no dilatation of the pupil was observed. Taken together, the highly poisonous nature of gelsemium may be attributed to the presence of gelsemicine, sempervine and possibly other amorphous bases which are under investigation.

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[3] Wormley, *Am. J. Pharm.*, 1870, **41**, 1.

[4] Sonnenschein, *Ber. Deutsch. Chem. Gessell.*, 1876, **9**, 1182.

[5] Gerrard, *Pharm. J.*, 1883, **13**, 641.

[6] Moore, *J. Chem. Soc.*, 1910, **97**, 2223.

[7] Thompson, *Pharm. J.*, 1887, **17**, 803.

[8] Cushny, *Arch. exp. Path. Pharm.*, 1893, **31**, 49.

[9] Sayre, *J. Am. Pharm. Assn.*, 1912, **1**, 458; 1914, **3**, 314. Stevenson and Sayre, *Ibid.*, 1915, **4**, 60. Sayre and Watson, *Ibid.*, 1919, **8**, 708.

[10] Chou, *Chin. J. Physiol.*, 1931, **5**, in press.

[11] Hou, private communication.

New York Section.

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5536

Regeneration of Virus Myxomatosum (Sanarelli) in the Presence of Cells of Exudates Surviving in Vitro.

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For a number of years the relation of viruses to cells has been under investigation in our laboratory. In pursuance of this study, attempts to cultivate the virus of infectious myxomatosis of rabbits in different kinds of tissue cultures were made. At the beginning of the work, bits of rabbit testicle suspended in a mixture of rabbit serum (1 part) and Tyrode's solution (3 parts) were used as a medium. Such preparations, however, proved unsuitable for the *in vitro* cultivation of the virus. Previous observations indicated that large mononuclear wandering cells are involved in this disease. Therefore, it seemed not unlikely that the inadequacy of the above methods of cultivation was referable to the failure of mobilization of these cells in excised tissues. To test this hypothesis, attempts to cultivate the virus in the presence of mononuclear cells obtained according to the method of Gay and Clark[1] were made.

A sterile mixture (6 cc.) of beef extract and gum acacia was injected into the right pleural cavity of a normal rabbit. Seventy-two hours later the cavity was opened aseptically and the fluid contents were aspirated. Heparin (1 cc. of a 1:1000 solution for each 10 cc. of pleural fluid) was added to the exudate to prevent clotting and the mixture was centrifuged for 5 minutes at low speed. The sediment was resuspended in Tyrode's solution and again centrifuged. Finally, the cells from 5 to 10 cc. of exudate were suspended in 2 cc. amounts of a mixture of rabbit serum (1 part) and Tyrode's solution (3 parts) and placed in 3 cm. Carrel flasks. To each flask with its 2 cc. of medium, 0.2 cc. of sterile tissue juice containing myxoma virus was added. The cultures were then incu-

[1] Gay, F. P., and Clark, A. R., *Arch. Path.*, 1926, **1**, 847.

bated at 37.5°C. for 3 or 4 days. Subcultures were accomplished by the transfer of 0.2 cc. of the incubated material to flasks containing 2 cc. of a fresh medium. In this manner the active agent has been carried through 20 subcultures. The titre of the virus in the first culture before incubation was 1:100. After incubation it was 1:10,000. In subsequent cultures the titre of the virus at the end of the period of incubation varied between 1:100,000 and 1:1,000,000.

In view of the fact that normal testicular tissue suspended in a mixture of serum and Tyrode's solution did not support the multiplication of the virus, a series of experiments was conducted to determine whether such tissue irritated by beef extract and gum acacia is capable of maintaining regeneration of the active agent. The irritant (1 cc.) was injected into the testicles of normal rabbits, and 72 hours later the irritated organs were removed and minced. Then bits of the minced tissue were suspended in a mixture of serum and Tyrode's solution. The medium placed in Carrel flasks was inoculated and handled as described above. In this manner the virus has been carried through 9 subcultures, and, in spite of a 10-fold dilution at the time of each transfer, the titre of the active agent at the end of each period of incubation has been between 1:100,000 and 1:1,000,000.

By means of the supravital technique, examinations of the exudates immediately after removal from the pleural cavities as well as after incubation in the cultures demonstrated that the primary cells were elements of the monocytic series. Active monocytes with small neutral red rosettes and all kinds of "stimulated" forms including the "coarse-granule" epithelioid type were regularly present in large numbers.[2] Histological studies of the irritated testicles revealed an infiltration of the interstitial tissue with large mononuclear cells which in supravital preparations were found to belong to the monocytic group.

These experiments demonstrate that the virus of infectious myxomatosis is capable of *in vitro* pullulation in the presence of cells surviving in liquid medium, and seem to indicate that cells of the monocytic series play an important rôle in the process. Experiments are now in progress to study the action of the virus in the presence of a variety of cellular elements in the hope of obtaining information regarding the specific susceptibility of different types of cells.

[2] Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 231.

5537

Rate of Decalcification and the Sites of Bone Lesions in Experimental Hyperparathyroidism.*

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A relatively rapid rate of mineral metabolism is associated with rapid bone growth in young animals. Our studies in acute and chronic experimental hyperthyroidism have shown a greater susceptibility to parathormone and a more rapid excretion of calcium in the young than in the adult.[1, 2]

Bauer, Aub and Albright[3] stated that in bone resorption the calcium of the trabeculae is "labile" and is drawn upon in the first instance, while the calcium of the compact bone ("the structural part of the bone") becomes available only "in the case of unusual body demands." Our studies suggest another conception: If we are to speak of labile calcium, it is the calcium in the regions of most active growth; if we are to speak of less readily available calcium, it is the calcium in the regions of less active growth.

We have examined the skeletons of about 150 young guinea pigs, 50 old guinea pigs, 25 young dogs, and 10 rats. The so-called characteristic lesions were severest and most easily produced in young animals suffering from acute and chronic hyperparathyroidism. The bones and portions of bones most affected were the metaphyses of the long tubular bones, and the cortex of the shaft, particularly near the epiphyseal cartilage plates; the costochondral junctions, and the cortex of the ribs used most in the respiratory act; the bones of the skull and of the lower jaw. The metaphyses of the slower growing short tubular bones showed relatively few lesions; the cortex of the shafts of these bones showed practically none. In the guinea pig, most epiphyses are already formed and the centers of ossification for the tarsal and carpal bones are present at birth. No specific lesions were found in these bones, at most a simple atrophy of their trabeculae was observed.

In adult animals, lesions were produced with greater difficulty. When definite, they followed doses so great that the lesions appeared

* Aided by a grant from the Herbert L. Celler Research Fund.

1 Bodansky, A., Blair, J. E., Jaffe, H. L., *J. Biol. Chem.*, 1930, **88**, 629.

2 Jaffe, H. L., Bodansky, A., Blair, J. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 174.

3 Bauer, W., Aub, J. C., Albright, F., *J. Exp. Med.*, 1929, **49**, 145.

in sites which escaped the effects of the smaller doses administered to the young (epiphyseal ends of the long tubular bones).

Thus, the typical lesions of experimental hyperparathyroidism which are merely secondary to the primary process of decalcification, occur especially in the regions of rapid growth. A higher rate of mineral metabolism in particular bones may also be associated with active use of these bones. However, a generalized thinning of the bones, without evidence of active resorption, is observed in the less susceptible bones.

The following formulation of the mechanism that determines the availability of calcium is suggested: Bone resorption and bone deposition are processes that go on in *all* bone constantly. When the rate of these processes is increased, it is increased in *all* bone. Both processes are more rapid in the regions of active growth, irrespective of anatomical structure. A mechanism is therefore indicated for rapid decalcification in those regions when a condition is present favoring an excess of resorption over deposition. In regions of less active bone growth, resorption and decalcification proceed more slowly under normal conditions, and a great stimulus is required to produce a considerable degree of resorption.

This conception makes intelligible the pronounced resorption of the trabeculae of the metaphyses, especially of the rapidly growing bones and the localization of the lesions in these regions; the trabeculae of all epiphyses under the same conditions are subject only to general thinning. It also explains not only the generally greater susceptibility of the bony skeletons of actively growing animals to parathormone, but also the quantitative difference in response of different bones, and of different portions of given bones, which is observed strikingly in young animals, but also to a certain degree in older animals.

It is suggested that the same principle applies in experimental rickets and scurvy, which show the localization of the so-called characteristic lesions in the regions of active growth; and in a number of clinical conditions (the so-called osteochondritides), which show localization of lesions in bones (and in regions of bones) of slow metabolic exchange and therefore of slow reparative capacity. Schmorl[4] in 1909 stated, in a discussion of clinical rickets, that the lesions appear earliest and are most severe in the regions of rapid growth.

[4] Schmorl, G., *Gesell. f. Natur. u. Heilk. in Dresden, Jahresbericht,* 1908-1909, 90, 18th sitzung.

5538

A Study of the Allergic Phenomena in the Central Nervous System.

CASPAR G. BURN AND KNOX H. FINLEY. (Introduced by R. Hussey.)

From the Department of Pathology, School of Medicine, Yale University.

These experiments were designed to study the allergic response of the central nervous system and to determine what part this phenomena might play in certain types of meningitis and encephalitis. Tuberculous animals were first employed because it is known that their tissues are sensitive to tuberculin.

Guinea pigs were inoculated subcutaneously in the groin with a known quantity of virulent human tubercle bacilli sufficient to give rise to an extensive generalized tuberculosis within 3 to 6 weeks. At varying intervals, up to 5 weeks, following the initial inoculation different animals had injected in their subarachnoid spaces by the way of the basal cisterns 0.3 cc. of a 1/10, 1/100, and 1/1000 dilution of old tuberculin. If the animals failed to develop fatal central nervous system disturbances they were killed by means of decapitation under ether anesthesia. Gross and microscopic studies were made of the brain and viscera. Cultures were prepared of the brains for the presence of the tubercle bacilli and other pathogenic organisms. As controls, both tuberculous and non-tuberculous animals were employed in each experiment. In this investigation 112 guinea pigs were employed, 42 of which were controls.

Following the subarachnoid injection of tuberculin the animals with advanced visceral tuberculosis showed definite and constant clinical central nervous system manifestations as well as a striking histological response in the leptomeninges. Restlessness, ruffled hair, progressive weakness, twitching and loss of sphincter control occurred 3 to 4 hours after the inoculation of tuberculin. Death usually occurred within 6 to 12 hours. Histological studies of the brains revealed an extensive polymorphonuclear exudate in the subarachnoid space. In the more advanced cases there was a definite perivascular extension and some glial proliferation around the vessels of the cerebral parenchyma. The acuteness and severity of the meningitis was proportional to the duration and extent of the generalized visceral infection; the course paralleled the skin test.

Similar experiments were performed in which dead and living tubercle bacilli were substituted for tuberculin. The onset and development of the meningitis apparently differed only slightly from the animals inoculated with tuberculin.

Tuberculous guinea pigs inoculated with glycerin broth by the way of the basal cisterns showed no clinical manifestations, but in some there was found a slight meningeal exudate on microscopic examination. Similarly, the nontuberculous animals inoculated with tuberculin and glycerin broth showed no response either clinically or microscopically. All animals inoculated in the neck muscles and other parts of the body with tuberculin revealed no evidence of activity in the meninges.

These experiments demonstrate that the meninges of tuberculous animals react to tuberculin, as well as dead and living tubercle bacilli, by an exudative response suggesting an allergic phenomenon. Other experiments are in progress.

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Fate of the Active Agent in the Chicken Sarcoma in Mixtures Containing Inhibiting Substances.

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We have previously described[1] the presence of a tumor inhibiting substance in the filtrate of the Rous chicken sarcoma and in normal chicken sera. It was found that this inhibiting substance is retained in the supernatant fluid when the tumor filtrate is brought to pH 4, while the active agent is carried down in the precipitate. In a more recent report, Murphy[2] and his associates report results that would seem to confirm these observations.

The fact that the activity of the agent can be inhibited by the supernatant fluid and sera made it of interest to ascertain whether the agent was actually destroyed in such mixtures or whether its tumor producing properties were merely inactivated. In order to answer this question it became necessary to determine if it is possible to recover the agent in an active state from these non-infective mixtures. The supernatant fluid used was prepared by adding an equal amount of a phthalate buffer solution at pH 4 to a 20% filtrate

[1] Sittenfield, M. J., Johnson, B. A., Jobling, James W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 517.

[2] Murphy, James B., Helmer, O. M., Claude, Albert, Sturm, Ernest, *Science*, 1921, **73**, 266.

of the Rous chicken sarcoma. After standing for 30 minutes, the mixture was centrifuged, and the supernatant fluid decanted, neutralized and concentrated *in vacuo* to ½ its original volume. To 2 cc. of the concentrated supernatant fluid was added 0.5 cc. of filtrate, and the mixture allowed to stand for 30 minutes at room temperature. Such a mixture when injected into chickens fails to produce tumors in 75% of the inoculations. If, however, the mixture is brought to pH 4, and the precipitate extracted at pH 8, the active agent is recovered, for the extract is now able to induce tumor growth. Similar results were obtained when blood was used instead of the supernatant fluid.

This experiment has been repeated several times, and the agent has been recovered in almost every instance. It is evident then that the tumor producing agent in these mixtures is not destroyed *in vitro* by the inhibitory substance. That we should fail to recover it in every instance is not strange, since the amount of the agent used in each mixture is not large, and we have never been able to extract all of it from the precipitate.

5540

Effect of Sulfhydryl Compounds on Regeneration in Podarke Obscura.

SERGIUS MORGULIS AND DAVID E. GREEN.

From the Marine Biological Laboratory, Woods Hole, Mass., and the Department of Biochemistry, University of Nebraska College of Medicine, Omaha.

From his studies on the toxicity of Pb ions in cell division in onion root tips, which he explained on the basis of the binding and eliminating of a SH-group, and from his experiments designed to show the effect of SH-groups on rate of growth depending on cell multiplication, as in growing onion root tips, Paramecia cultures, and healing of wounds, Hammett[1] postulates his theory that the SH is the "mitotic hormone" and the wound hormone or essential chemical factor in cell proliferation following trauma.

With the thought that a specific mitotic hormone would at least accelerate, if not also actually increase, regeneration since the latter depends on cell division and proliferation in its earlier stages, we performed a series of experiments on regeneration of the polychaete

[1] Hammett, F. S., *Protoplasma*, 1929, 7, 297.

worm, *Podarke obscura.* This animal was chosen because one of us (S. M.) has had extensive experience with this material and had previously made a long study of its regenerative process.[2]

The procedure was simple. Under a magnifying glass we cut away the posterior portion of the worm (which, by the way, never regenerates a head) aiming always to effect the severance at practically the same relative level. The cut must be made clean so that the wound closes up smooth and without any adhering shreds of necrotized tissue. All these details of operation are extremely important, if one is to insure a normal progress of regeneration. The same number of operated worms were distributed in a series of fingerbowls containing 90 cc. sea water with or without the tested reagents. In some of the experiments we followed a preferable course of leaving the worms in pure sea water until the wound had closed over and the first signs of initiation of the regenerative process appear. For a statistical study it is better if the reagents whose effect is being studied are applied to animals all of which have an even start. This eliminates from counts specimens which had begun their regeneration with some handicap and continue to lag behind. The control animals of each series were kept in pure sea water, while the others were in sea water containing various concentrations of the studied sulfhydryl compound. The water was changed daily and the solutions were made up fresh every day. The fingerbowls pertaining to one series of experiments were stacked together and kept in diffuse light, thus insuring uniformity of temperature and light conditions.

The rate of regeneration was determined by the number of new segments which were formed. The worms were examined daily with a binocular microscope. One familiar with the manner in which these animals regenerate can distinguish the new segments at a very early stage of their formation. The study, to be limited to the process of cell proliferation, can not be much prolonged, because after the first few segments have been formed the growth by increase in size becomes the predominant feature.

The sulfhydryl concentration of the various test solutions was determined daily. A solution of the particular sulfhydryl compound was prepared and its SH concentration was measured by treating this with an excess of a standard iodine solution and titrating back the unused amount of iodine with 0.01 N Sodium thiosulfate. The desired concentration of SH was then produced by proper dilution with sea water. The further dilutions in the finger-

[2] Morgulis, S., *J. Exp. Zool.*, 1909, **7**, 595.

bowls were effected simply by arranging the series in such a way that the successive concentrations formed a geometrical progression.

We studied the effect on the rate of regeneration of the following compounds: thio-p-cresol, thio-phenol, thio-glycollic acid, and cystine. The former 2 compounds were also controlled by means of p-cresol and phenol. Cystine was used because from Hammett's experiments, although he seems to attribute to SH the hormonal

TABLE I.
Percent of total number of animals.

Number days	Stage regeneration	Control sea water	Thio-p-Cresol Mgm. S per cc. of sea water				p-Cresol Gm. per cc. sea water			
			2.6×10^{-6}	2.6×10^{-7}	2.6×10^{-8}	2.6×10^{-9}	1×10^{-7}	1×10^{-8}	1×10^{-9}	1×10^{-10}
3rd	1 Segment	92	0	50	75	90	0	50	90	100
4th	2 "	75	Dead	29	64	54	0	30	45	90
	3 "	25		0	0	18		10	0	10
6th	4 "	17		38	64	90	0	33	10	10
	5 "	57		0	9	0		43	78	10
8th	5 "	33		60	20	45		37	30	10
	6 "	33		0	30	23	0	13	30	50

TABLE II.
Percent of total number of animals.

Number days	Stage regeneration	Control sea water	Thio-glycollic Acid. Mgm. S per cc. of sea water							
			2.5×10^{-4}	2.5×10^{-5}	2.5×10^{-6}	2.5×10^{-7}	2.5×10^{-8}	2.5×10^{-9}	2.5×10^{-10}
2nd	1 Segment	100		100	100	100	100	100	100
3rd	2 "	100		100	100	88	94	90	100
4th	3 "	100	100	100	100	100	100	100	100
5th	5 "	58	0	28	55	58	55	60	56
6th	6 "	83	0	29	59	70	72	80	62
7th	7 "	67	Dead	33	41	56	73	61	62

property, it is obvious that he finds both the reduced and oxidized sulfur group effective.

The results are recorded in the accompanying tables, giving for each day the percentage of animals which have reached a certain maximum number of segments in the control and in the various concentrations of the tested solutions. Since those animals lagging behind are thus not included, the percents do not always add up to 100.

The reported data hardly require comment. As can be seen, there is no evidence of a cumulative advance in the regeneration of experimental animals as compared to the controls. In many cases the concentrations of the tested SH compounds were such as to be even toxic. Even in non-toxic concentrations, however, we found either that worms in the test solutions actually regenerated slower than the controls or at best about the same rate. If a slight advantage seems to appear one day, it is alternated with a disadvantage the next.

The sharp discrepancy between our results and those reported by Hammett calls perhaps for some critical evaluation. Owing to limitation of space it will be merely pointed out that Hammett's method of calculation applied to his experiments with the SH compounds is theoretically erroneous. The difference between the control and test root tips is not expressed on the basis of the absolute findings but as a difference of the percent of increment in both the control and treated roots. This tends to grossly exaggerate the actual differences. Considering that the changes reported are not sufficiently striking to justify the claim that sulfhydryl compounds in general constitute the mitotic hormone, they really become quite insignificant when recalculated as the percent of increment rather than as the *difference in percents of increment,* as Hammett does.

The counting of mitotic divisions in growing root tips is also subject to the serious criticism that since mitosis is not a continuous process but occurs in cycles one must be sure, of course, that the comparison is made at exactly the same phase of the cycle in both control and test objects.

The experiments with Paramecia are likewise very inconclusive. No check has evidently been kept on the pH of the culture, which is a serious omission. The differences in the *number of divisions* which the untreated and treated Paramecia undergo are not sufficient to draw any conclusions, considering the extreme variability of the material even under ordinary conditions. Finally, the experiments with thio-cresol[3] which seem to show its stimulating action

[3] Reimann, S. P., *J. Am. Med. Assn.*, 1930, **94**, 1369.

upon cell proliferation as manifested in the healing of extensive wound surfaces treated with this substance fail to take into account that the use of the dilute alcohol as solvent may be actually responsible for the good clinical results. Surgeons are well familiar with the beneficent effect of dilute alcohol wet dressings.

Since this paper was written two articles came to my attention which should be mentioned here. Gaunt[4] studying the division of fresh water snail eggs under the influence of cysteine failed to find any stimulating action. Secondly, Voegtlin and Chalkley[5] in a splendid investigation on the effect of reduced and oxidized glutathione on the division of *Amoeba proteus* have brought forth evidence of a stimulating action on *nuclear* division, which, however, is conditioned by the state of "maturation" of the cell, *i. e.,* upon some purely intrinsic factors, the nature of which is entirely unknown. These results are extremely significant, but they have little bearing upon Hammett's simplified scheme of mitosis of which SH is the key.

Summary. Neither thio-p-cresol, thio-phenol, thio-glycollic acid or cystine accelerates the process of regeneration in the *Podarke obscura.*

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Effect of Oestrin and Lutein Combinations on Uterus of the Mouse.

DAVID I. MACHT AND ARTHUR E. STICKELS.

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It is well established that the physiological effects of the oestrin or follicular hormone of the ovary, when injected into various animals, will cause enlargement of the uterus. Allen and Doisy have very recently reviewed literature on the subject.[1] Advantage has been taken of this property of the ovarian hormone in a most interesting way by A. C. Siddall for the diagnosis of pregnancy and also for the evaluation of commercial ovarian products.[2, 3] From a study of a large number of mice, Siddall found that the ratio of the weight of

[4] Gaunt, R., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 660.

[5] Voegtlin, C., and Chalkley, H. W., *Public Health Reports,* 1930, **45**, 3041.

[1] Allen and Doisy, *Physiol. Rev.,* 1927, **8**, 60.

[2] Siddall, *J. Am. Med. Assn.,* 1928, **90**, 380.

[3] Siddall, *J. Am. Med. Assn.,* 1928, **91**, 779.

the uterus of a non-pregnant, mature white mouse to its body weight is usually over 400. When material containing the ovarian hormone is injected into such mice, hypertrophy of the uterus is produced so that the ratio of the uterine weight to the total body weight becomes less than 400. The present author has made a study of a series of normal mice and determined the ratio of the weight of the uterus in non-pregnant condition to the weight of the animal and was able to confirm Siddall's findings in this respect. In all cases the ratio was over 400 and, indeed, was often very much higher. Of 60 normal mice, the average ratio of the weights of the uteri to the body was 596, the lowest reading being 404 and the highest, 970. Of the 60 readings, 19 were between 404 and 500, 19 between 500 and 600, 3 between 600 and 700, 8 between 700 and 800, 7 between 800 and 900, and 4 between 900 and 960. These findings, together with the much more extensive findings first described by Siddall, suggested the present investigation.

A series of healthy, mature, non-pregnant white mice, weighing from 15 to 20 gm. each, and occasionally a little more, were divided into 2 groups. One group was injected daily with a standardized solution of the follicular hormone obtained on the market. The dosage injected daily varied from 1 to 2 Allen-Doisy mouse units. Another group of the same series of mice was injected with the same quantity of the follicular hormone together with an aqueous extract of corpus luteum prepared in these laboratories by a method previously described.[4] At the end of 10 days or more, the animals

TABLE I.
Injections of Oestrin Alone and Oestrin Plus Lutein
(October 24 to November 15, 1929).

	Oestrin alone, 0.2 cc. daily			Oestrin, 0.2 cc., plus lutein, 0.5 cc. daily		
	Weight of Body gm.	Weight of Uterus mg.	Ratio	Weight of Body gm.	Weight of Uterus mg.	Ratio
I	20,000	50	400	22,700	25	908
II	22,200	50	444	19,500	20	975
III	25,800	65	397	24,700	45	549
IV	27,200	70	389	19,000	20	950
V	22,000	170	129	27,200	85	320
VI	20,150	55	366	16,800	30	560
VII	20,840	55	379	21,450	30	715
VIII	20,550	55	374	20,950	30	698
IX	25,560	125	204	23,400	45	520
X	22,500	110	205	21,550	40	539
	226,800	Average	282	217,250	Average	587

[4] Macht, Stickels and Leach, PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 152.

were killed and carefully weighed. They were then cut open; the uteri were carefully dissected out and weighed on the chemical balance; and the ratio of the weight of the uteri to the total body weight was determined. Ten series of these experiments were performed; and the findings with such injections are illustrated in Tables I to

TABLE II.

Mouse	A Injected Oestrin Alone, 0.5 cc.			B Injected Oestrin, 0.5 cc., Plus Lutein, 0.5 cc.			C Injected Oestrin, Plus Predigested Lutein, Same as in B		
	Weight of Body gm.	Weight of Uterus mg.	Ratio	Weight of Body gm.	Weight of Uterus mg.	Ratio	Weight of Body gm.	Weight of Uterus mg.	Ratio
I	12,770	32	399	18,780	40	469	20,380	45	453
II	11,500	25	460	18,860	45	419	21,550	35	616
III	23,000	125	184	25,030	62	403	22,120	50	442
IV	23,825	75	317	20,730	40	513	19,070	35	545
V	20,300	70	290	26,050	65	401	21,900	45	487
VI	25,200	125	202	23,800	58	410	21,680	40	542
VII	24,100	110	215	17,030	40	426	20,810	35	594
VIII	16,400	65	252	17,050	40	427	18,830	45	418
IX							20,080	50	402
X							18,780	45	417
	Average		289	Average		434	Average		492

III. In Table I are recorded experiments made with 0.2 cc. of a diluted follicin solution, equivalent to about one Allen-Doisy mouse unit of follicin, and 0.5 cc. of lutein, equivalent to 100 mg. of the dry corpus luteum gland substance. It will be seen that after the injections of oestrin alone, definite and decided hypertrophy of the uteri was found, as indicated by the ratio of the uterine weight to the total body weight. These findings could be easily seen on inspection of the organs with the naked eye. On the other hand, when the same doses of oestrin were injected together with an extract of corpus luteum, no hypertrophy of the uterus occurred and the figures obtained for the ratio of the 2 weights were over 400. In another series of 10 mice each, a different preparation of follicular hormone was used and similar results were obtained. The oestrin series gave ratios of 248 to 390 with an average of 295, and oestrin plus lutein gave ratios of 340 to 516 with an average of 465.

In Table II are recorded another series of experiments with 3 groups of mice. In Group A, the oestrin alone was injected, in doses of 0.5 cc., or about 2 mouse units. In Group B, the same amount of oestrin was injected together with 0.5 cc. of corpus luteum extract; and in Group C, the oestrin was injected together with a corpus luteum extract prepared from *predigested* corpus luteum gland substance. Here again, it will be noted that the oestrin alone produced hypertrophy of the uteri, as indicated by the low ratio of the uterine and body weights. In Group B, the combination of oestrin and lutein did not produce hypertrophy of the uterus, and in Group C, the results obtained with *predigested* lutein were the same as those obtained with ordinary lutein solution. Other series of experi-

TABLE III.
Feeding Experiments.

	Oestrin Alone			Oestrin Plus Lutein		
	Weight of Body gm.	Weight of Uterus mg.	Ratio	Weight of Body gm.	Weight of Uterus mg.	Ratio
I	21,600	55	393	21,840	30	728
II	27,000	70	357	24,440	40	611
III	28,000	80	350	21,000	25	840
IV	28,100	80	352	18,370	22	835
V	23,600	60	393	17,820	20	891
VI	24,700	65	380	20,370	25	815
VII	27,150	70	388	24,620	40	616
VIII	20,520	55	380	13,580	19	714
IX	13,940	35	398	15,900	20	795
X				22,500	35	898
		Average	377		Average	774

ments, performed in the same way as those illustrated in the tables, gave similar results. It would therefore seem that an injection of corpus luteum extract tends to counteract the hypertrophy-producing effect of the oestrin or follicular hormone in mice.

The authors furthermore made studies of a similar nature in regard to the feeding of follicular hormones and extracts of corpus luteum. This was done by sprinkling on a slice of bread a solution of oestrin alone and of oestrin together with lutein and feeding the different groups of mice. After 10 days very little effect was noted, but, on continuing the feeding experiments for periods of from 2 to 5 weeks and examining the animals after killing them, it was found that there was a distinct difference in the size of the uteri and in the ratios obtained between uterine and body weights. In Table III is illustrated one series of such experiments in which oestrin alone in doses of from 2 to 5 mouse units was devoured by each mouse daily, on the one hand; and, on the other, in which the same quantity of the oestrin was fed with 0.5 cc. of the lutein solution on bread. It may be added that injections of corpus luteum extracts alone produced no appreciable change in the size of the uteri of control mice. Investigations are being continued and it is hoped that this method may furnish a practical way of evaluating the physiological activity of corpus luteum preparations.

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Reaction of Blepharisma to Golgi Impregnation Methods.

IMOGENE MOORE. (Introduced by L. L. Woodruff.)

From the Osborn Zoological Laboratory, Yale University.

From a study of the normal and regenerating contractile vacuoles *in vivo*, evidence was obtained that the normal contractile vacuole in *Blepharisma undulans* is not a permanent cell organ, but a system of temporary, potentially independent fluid vacuoles.[1] In view of the fact that conflicting opinions concerning the permanence of contractile vacuoles have arisen from the failure of investigators to study both living and stained preparations, the above conclusions have been tested by a study of fixed material, derived from the pedigreed cultures. The Nassanov methods and the original Kolatschev technique were employed, not only because of their proved

[1] Moore, I., *Anat. Rec.*, 1930, **47**, 346.

success in demonstrating contractile vacuole structure, but also because by their use the Blepharisma vacuole could be examined in the light of the Nassanov homology.

In the several series of preparations which were made, Paramecia, mixed with the Blepharisma prior to fixation and treated identically step by step, were used as controls. Although the contractile vacuoles of the Paramecia showed the characteristic blackening, those of the Blepharisma failed to do so. In general, where osmication was continued until the whole cytoplasm was darkened, the food and contractile vacuole walls in the Blepharisma were likewise blackened, but upon differentiation of such preparations in turpentine, these walls became bleached as quickly as the surrounding cytoplasm. Particularly careful examination of the preparations showing darkened vacuolar walls for any sign of the old primary vacuole wall which, if present, should form a small dark mass close to the anal spot, revealed no trace of that structure. However, sparsely distributed through the entire endoplasm of the Blepharisma were small, globular bodies which did show a typical Golgi impregnation. These bodies with their osmiophile cortices and osmiophobe centers resembled both in structure and staining reaction Golgi bodies which have been described for several other Protozoa.[2] Particularly does the situation in Blepharisma recall that in *Amoeba proteus,* Pallas, as described by Brown.[3] However, absolutely no evidence indicating that in the osmiophile bodies lay the origin of the contractile vacuole was found in Blepharisma.

Accordingly, further proof of the temporary character of the Blepharisma contractile vacuole has been obtained. Moreover, since this vacuole reacts negatively to the Golgi techniques, it must be considered as an exception to the Nassanov homology.

My thanks are due to Prof. J. Bronté Gatenby for suggestions concerning the Golgi methods as well as for critical examination of the osmic preparations, and to Prof. L. L. Woodruff, under whose direction the entire research is being carried out.

[2] Bowen, R. H., *Anat. Rec.*, 1928, **40**, 225.

[3] Brown, V. E., *Biol. Bull.*, 1930, **59**, 240.

5543

Specificity of Reactions Produced by Injection of Urine from Pregnant Cows into Immature Female Guinea Pigs.*

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Recent experiments have shown that the anterior hypophysis, in addition to its primary growth inducing function, has a secondary, apparently specific effect upon the sex organs. Under the influence of transplants of anterior hypophysis taken from various mammals, immature mice and rats have been brought to precocious sexual maturity (Smith and Engle,[1] Zondek and Aschheim[2]). One of the most striking effects produced by the anterior hypophyseal transplants has been the luteinization of follicles in the ovaries of the female host animal. Within 2 days after the first transplantation the ovaries began to show hyperemia accompanied by progressive growth of follicles. About 2 to 3 days later a distinct luteinization process was initiated in a number of follicles, which changed into atretic corpora lutea. Similar phenomena had been previously recorded by Evans and Long[3] after intraperitoneal injections of fresh anterior hypophyseal substance. These authors believe the whole reaction to represent "a powerful, specific stimulus to lutein cell transformation". This interpretation of specificity has been expanded by later investigators who attribute all the changes, caused by the transplants upon sex glands, to a separate "extra-gonadal" or "sex maturing" anterior pituitary hormone. Through the discovery of this particular hormone, in large quantities, in the urine of pregnant women, Aschheim and Zondek[4] developed an important method for diagnosing pregnancy. Injections of urine from pregnant women given to immature female mice resulted as with anterior hypophyseal transplants in the development of atretic corpora lutea and of hemorrhagic spots in the ovaries. The application of this test gave very satisfactory results with human urine. On the

* This work has been aided by the Committee for Research on Sex Problems of the National Research Council and by the Mount Hope Farm of Williamstown, Mass.

[1] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, **40**, 159.

[2] Zondek, B., and Aschheim, S., *Arch. f. Gynak.*, 1927, **130**, 1.

[3] Evans, H. M., and Long, T. A., *Proc. Acad. Sciences*, 1920, **8**, 38.

[4] Aschheim, S., and Zondek, B., *Klin. Wochenschr.*, 1928, **7**, 1404.

other hand, tests made with the urine of pregnant cows, and of other mammals have been reported as negative.[5, 6]

In the course of experiments with cows, carried out with the cooperation of Mount Hope Farm,† we obtained material for the study of the effect of urine injections upon immature female guinea pigs.

Each animal received 6 injections of 1-2 cc. of cow's urine on 2 consecutive days. Vaginal smears were taken the third and fourth days and then the animals were killed and autopsied.

The ovaries of test animals, which received injections of pregnant urine did not show formation of corpora lutea or the large, typical hemorrhagic spots. On the other hand there was a definite increase in the vascularization of follicles and in their atretic degeneration. A number of follicles evidently began to grow, but, before reaching maturity, their theca interna became highly vascularized and caused their early disintegration. This excessive vascularization of medium sized follicles was one of the most outstanding features of the ovarian changes.

Outside of the follicular theca this reaction was less pronounced. Blood extravasations were observed but not as extensive nor typical as those described in mice.

The vascular phenomena, which appear after the injection of pregnancy-positive urine are not limited to the ovary or the genital tract. Other glands, like the adrenals, the thyroids, and the hypophysis display certain vascular changes.

These changes are particularly evident in the adrenals. In the most typical cases, the large vessels of the medulla and those of the reticular zone of the cortex become conspicuous and show a characteristic state of turgescence and a regularity in their general conformation. At the same time certain morphological changes are apparent in the various zones of adrenal cortex and especially in the reticularis. This last zone is greatly stimulated and shows a pronounced hypertrophy. Its cells are well defined with normal, rounded nuclei and numerous aggregations of secretory granules.‡

[5] Aschheim, S., *Am. J. Obst. and Gyn.*, 1930, **19**, 335.

[6] Allan, H., and Dickens, F., *Lancet*, 1930, **238**, 39.

† Experimental work related to various problems of reproduction has been conducted for the last 2 years at the Mount Hope Farm at the suggestion of Mr. E. Parmalee Prentice.

‡ These changes appeared in a large number of guinea pigs injected with pregnancy-positive urine. They were absent in control animals, injected with normal (non-pregnant) urine. A disturbing factor has been found in the occasional infection of some of the injected animals. This caused a congestion of the adrenal cortex and an apparent stimulation of the two external zones, the

These effects upon the adrenals and the vascular system may offer an explanation for the changes occurring in the ovaries after injections of urine from pregnant women. When an immature female rodent is injected with such urine its ovarian follicles and especially their theca interna become highly vascularized. Under normal conditions the process of the vascularization of the follicles ocurs at the completion of their ripening and prior to their rupture and their transformation into corpora lutea. In the injected animals the vascularization occurs prematurely and its effects are different according to the size of the affected follicles. A large, almost mature follicle, may turn into an atretic corpus luteum or may rupture and form a typical corpus luteum. A smaller immature follicle will become atretic and disintegrate or may become hemorrhagic through the rupture of the abnormally congested vessels of its theca. The transformation of granulosa or theca cells into luteal elements is an inherent property of these cells and may be completed under the influence of certain external stimuli.

In view of these considerations, one may explain why various species of rodents react differently to the injection of pregnancy-positive human urine. In animals with a very short sex cycle, such as mice or rats, in which the ripening of the follicles occurs rapidly, the vascular stimulation of the follicles, running its course within 4 to 5 days, leads to the development of large follicles and atretic corpora lutea. In animals having a longer cycle, such as the guinea pigs, in which the follicular growth is completed at a much slower rate, the vascularization of the theca does not induce the full development of ripe follicles and their transformation into corpora lutea. The stimulation here results in a relative growth of follicles leading to their final atretic degeneration. In rabbits, in which the absence of a spontaneous ovulation permits the use of larger animals for pregnancy tests, the formation of corpora lutea or hemorrhagic follicles can be induced within 24 hours after the injections of pregnancy-positive urine.[7] This is made possible by the presence of large follicles within the ovaries.

The existence of a specific hormone, the so-called "sex-maturing hormone", which is supposed to affect the sex glands primarily and to cause a luteinization of the ovarian follicles as well as a precocious sex-maturity becomes highly improbable in the light of these investigations. This active substance, whatever it is, seems to have

glomerulosa and fasciculata, with an increase in mitotic figures. This reaction could be recognized as being of pathological nature and should not be confused with the above described normal reaction.

[7] Friedman, M. A., *Am. J. Physiol.*, 1929, **90**, 617.

a general effect upon the vascular system, possibly through the adrenal glands, causing secondary effects in various organs, including the ovaries. The need for a stimulation of the glandular system and the growth of vascular tissues like the placenta during pregnancy might account for the existence of a general reaction of this type.

5544

Is Levulose Converted to Dextrose in the Process of Absorption from the Small Intestine?

G. E. BURGET AND PHILIP H. MOORE.

From the Department of Physiology, University of Oregon Medical School.

A number of experiments were carried out by injecting levulose into closed loops of small intestine in normal unanesthetized dogs. After one hour the remaining loop fluid was aspirated and its total reducible sugar determined. A polariscope reading was made on a cleared specimen and a levulose determination made by the colorimetric method of Corley.[1] No dextrose was indicated in the aspirated fluid by these methods. However, this did not exclude the possibility of conversion in the mucosa with none demonstrable in the loop fluid. It became necessary for us to know whether or not levulose was present in the mesenteric vein from the loop during absorption. Forty-five minutes after giving levulose by loop the animal was anesthetized, abdomen aseptically opened, mesenteric vein from the loop exposed, and 10 cc. of blood withdrawn. A similar amount of blood was taken from the heart immediately following. Blood sugar was determined in each specimen and both were tested for levulose. In 5 experiments levulose was found in the mesenteric blood with the systemic blood negative. The mesenteric blood sugar was greater than that of the systemic blood by the amount of levulose found in mesenteric blood. Since the amount of levulose given (1.5 to 2 gm.) was shown not to cause a rise in systemic blood sugar in separate experiments, these findings indicate that no levulose is converted to dextrose in the process of absorption from the small intestine.

[1] Corley, R. C., *J. Biol. Chem.*, 1929, **81**, 81.

5545

Effect of Irradiation on Electrokinetic Potential, Agglutinability, Lysis and pH of Escherichia Coli Suspensions.*

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(Introduced by R. Adams Dutcher.)

From the Department of Agricultural Biochemistry and the Division of Bacteriology, Department of Dairy Husbandry, Pennsylvania State College.

From a study of the effect of irradiation of aqueous suspensions of *Escherichia coli* with the rays from a B carbon arc, the following conclusions, which harmonize with our hypothesis concerning electrokinetic potential as a measure of activity, stimulation, injury, recovery and death[1] have been drawn:

1. Storage and exposure to atmosphere of room in which the irradiation was carried out were not the cause of the change in electrophoretic velocity noted after irradiation, nor did they change the ability to be agglutinated.

2. Insertion of Corex A glass filter lessened the action of the ultraviolet radiation considerably. This suggests that the shorter wave lengths are more bactericidal and charge-reducing.

3. It has been shown [using both Northrop-Kunitz (maximum and actual values) and Falk capillary cells] that irradiation, if of sufficient duration, produces a decrease in negative charge which accompanies death.

4. Data obtained with the Northrop-Kunitz cell (both maximum and actual velocity) indicate an initial stimulative action of the ultraviolet radiation, which makes itself felt in an increase of negative charge. This increase of charge was not often observed with the Falk capillary cell.

5. Short time irradiation, which merely stimulates or injures, permits of a return toward normal of electrokinetic velocities. Long time irradiation, which kills, produces a lasting effect.[2]

6. The time after irradiation at which comparative electrophoretic velocity readings are made must be carefully controlled if the irradiation is of brief duration.

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† The authors wish to acknowledge the invaluable assistance of G. W. Sharpless, K. P. Dozois, E. C. Holst, R. L. Ferguson, and Dr. W. P. Davey.

[1] Tittsler and Lisse, *J. Bact.*, 1928, **15**, 105.

[2] Osterhout, Injury, Recovery and Death. Lippincott, Phila., 1922.

7. Bacterial suspensions having practically the same charge can be prepared over a long period of time from different generations of the same organism.

8. Electrophoresis studies are more sensitive than the usual agglutination studies for detecting the effect of irradiation on the charge of *Esch. coli.*

9. The sequence of decreasing electrophoretic velocities is the same as that of increasing agglutinability except in the case of short (return to normal follows) and very long (lysis sets in) time irradiation.

10. The introduction of a Corex A filter practically causes this sequence of agglutinability to disappear.

11. Irradiation produced lysis which was greater the longer the irradiation.

12. Irradiation produced an increase in pH of the unrayed aqueous suspension whose pH was approximately 6.1. Since such change was also observed when the water itself was irradiated, it suggests the use of recently boiled water in future work.

13. Similar work, using 500 watt Mazda bulbs and Falk cells, showed eventual reduction of charge (which was preceded by a stimulatory increase), and evidence of lysis as indicated by clearing of the suspension. To accomplish similar effects, much longer times of irradiation were necessary than with ultraviolet rays.

14. X-rays produced no changes in electrophoretic velocities, nor did they produce bactericidal action.

Further evidence in favor of our hypothesis concerning electrokinetic potential as a measure of activity, stimulation, injury, recovery and death, is obtained from our work with the nitrogen fixing organism, *Rhizobium meliloti.* In general those cultures (history known) which were high nodule producers or high nitrogen-fixers had a higher negative potential than those of low abilities (Zucker[3]). An objection to the hypothesis is to be found in the work on heat killing of bacteria.[4]

Since this work was begun, a number of statements suggesting these findings have appeared. Norton[5] suggested that the changes that bacteria suffer when exposed to ultraviolet rays are accompanied, perhaps preceded, by changes in the electrical charges of the bacteria. Beaver and Muller[6] stated that red gold sols change to

[3] Zucker, *J. Bact.*, 1929, **17**, 18.

[4] Winslow, Falk, and Caulfield, *J. Gen. Physiol.*, 1923, **6**, 177.

[5] Norton, Newer Knowledge of Bacteriology and Immunology, Jordan and Falk. Univ. of Chicago Press, 1928, 374.

[6] Beaver and Muller, *J. Am. Chem. Soc.*, 1928, **50**, 304.

blue on exposure to ultraviolet rays, but by prolonged irradiation they are peptized to stable red sols. Falk and Reed,[7] working on the alterations in red blood cell electrophoretic potential produced by direct irradiation of blood *in vivo* reported a slight decrease of potential difference. Mayer[8] says, "So far no attention has been paid to the importance of the nature of the electrical charge on a substance that is irradiated by ultraviolet light. A study of the effect of light on body tissues from this point of view promises interesting results."

A detailed account of these findings will appear in a technical bulletin of the Pennsylvania Agricultural Experiment Station. For progress reports see [9, 10].

5546

Effect of Basic Diets on the Rate of Incisor Tooth Growth.

WILLIAM G. DOWNS, JR.

From the Department of Pathology, Yale Medical School.

In a series of studies on the effects of diet on bone healing and growth and tooth development and growth of the albino rat, it was decided to measure the rate of growth of the incisor teeth after the method of Addison and Appleton.[1] The following is a preliminary report.

The animals were males of approximately 6 months of age, and weighed between 190 and 300 grams, averaging 243 grams. All the animals on each type of study were kept under as nearly identical conditions as possible. A group of over 200 controls were fed on the standard diet of Smith and Moise.[2]

Other groups of 140 animals, were fed on a high-protein diet, on a high-fat diet, on a high-carbohydrate diet.[2] Similar groups were then given the standard diet with the salt mixture varied so as to result in a low-total salt diet in one instance, a (nearly) calcium-free

[7] Falk and Reed, *Am. J. Physiol.*, 1926, **75**, 616.

[8] Mayer, Clinical Application of Sunlight and Artificial Radiation. Williams and Wilkins, Baltimore, 1926, 355.

[9] Lisse, Bull., 1928, **230**, 6; 1929, **243**, 6; 1930, **258**, 8. *Penn. Agr. Exp. Sta. Ann. Reports.*

[10] Tittsler and Dozois, Bull., 1930, **258**, 28. *Penn. Agr. Exp. Sta. Ann. Reports.*

[1] Addison, W. H. F., and Appleton, J. L. T., Jr., *J. Morph.*, 1915, **26**, 43.

[2] Smith, Arthur H., and Moise, Theodore S., *J. Exp. Med.*, 1924, **43**, 13.

diet in another, and a (nearly) phosphorus-free in a third. These salt mixtures were made according to the method of Osborne and Mendel,[3] and the fundamental dietary values in each case have been accurately determined by these earlier workers. All animals in each group received a similar and adequate quantity of the various vitamines.

The animals were allowed to remain on the diet for a full week before any observations were made. Groups of 7 animals were then killed at 3-day intervals to 51 days, then at 60-day, 90-day and 6-month periods. The results of radiographic and histological studies on the teeth and jaws of these animals will be published later.

The rate of tooth growth and averages were obtained of the rate of tooth growth on each type of diet. In many instances 4 measurements were taken of each animal at each observation and the result of these 4 averaged. It was found, however, that there was so little variation in these measurements that this was not systematically followed. Measurements were made on a strict 7-day basis, even to the point of making the observations at as nearly as possible the same time in the day. (Table I.)

TABLE I. Rate of Tooth Growth.

	mm. per week
Normals	2.78
High Fat	3.05
High Carbohydrate	3.50
High Protein	3.21
Low-total Salt	2.14
PO_4 Free	2.15
Ca Free	2.30

It had been determined by Addison and Appleton, that the average rate of lower incisor tooth growth in the normal adult albino rat was 2.8 mm. per week. This was checked by our control series and our results very closely coincided with this figure. With that as a base line for normals, the observations on the other types of diets were made. The average rate of growth on a particular diet is based on not less than 100 observations taken at similar periods for each group.

[3] Osborne, Thomas B., and Mendel, Lafayette B., *J. Biol. Chem.*, 1918, **34**, 131.

5547

Action of Certain Bacteria on Uric Acid and Its Substitutes.

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From the Institute of Pathology, Western Reserve University, Cleveland, Ohio.

Morris and Ecker[1] demonstrated that uric acid is destroyed in fecal infusions under conditions that indicate activity of microorganisms. Various bacteria were studied to determine their ability to utilize uric acid. It was found that 6 strains of *B. aerogenes* grew and utilized uric acid in Koser's medium and that the *Bacterium acidi urici* (Ulpiani) which was isolated from chicken excreta readily destroyed all the uric acid employed within 30 hours. No uricolytic enzyme, however, was extracted from the organisms.

These observations led to the present study of whether or not the above named organisms are capable of attacking (a) 1-monomethyl uric acid, (b) 3-monomethyl uric acid, (c) 3-9-dimethyl uric acid, (d) 1-3-dimethyl uric acid, (e) 1-3-7-trimethyl uric acid, and a study of the selective destruction of uric acid in a mixture containing the substituted uric acids.

The organisms were implanted in the Benedict and Hitchcock[2] phosphate standard (containing 9 gm. of Na_2HPO_4-12 H_2O; 1 gm. NaH_2PO_4-H_2O; and 200 mg. uric acid per liter). The pH was 7. The substituted uric acids were added in colorimetric equivalents since they gave less color than the uric acid itself. We used for each mg. of uric acid: 1 mg. of 1-monomethyl uric acid, 4 mg. of 3-monomethyl uric acid, 7 mg. of 3-9-dimethyl uric acid, 1.2 mg. of 1-3-dimethyl uric acid, 14 mg. of trimethyl uric acid.

All the culture tubes contained 5 cc. of the media, sterilized at 15 lb. for 15 minutes. Inoculations were made with the Pasteur pipette using 2 drops of a saline suspension made from a 24-hour culture of the organism on slant agar. The organisms were allowed to grow for various periods of time up to 14 days at 37°C. and all determinations were made by the Benedict and Franke[3] method.

Since sterilization might destroy a considerable amount of the uric acid and its substitutes, the amount destroyed in 15, 30 and 45 minutes at 15 lb. pressure, was determined. The results were as follows:

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[1] Morris, J. Lucien, and Ecker, E. E., *J. Inf. Dis.*, 1924, **34**, 592.

[2] Benedict, S. R., and Hitchcock, E. H., *J. Biol. Chem.*, 1915, **20**, 619.

[3] Benedict, S. R., and Franke, E., *J. Biol. Chem.*, 1922, **52**, 387.

TABLE I.
Showing the % destruction of uric acids and its substitutes in the autoclave.

Uric acids	Time in minutes		
	15	30	45
Uric	9.7	15.9	24.0
1-monomethyl	12.5	19.7	23.4
3-monomethyl	14.0	14.1	14.9
3-9-dimethyl	9.0	4.0	11.0
1-3-dimethyl	15.6	16.2	15.6
1-3-7-trimethyl	6.0	10.0	7.0

The usual 15 minutes exposure at 15 lb. pressure, therefore, does not destroy more than 15% of the compounds employed.

The *Bacterium acidi urici* of Ulpiani destroyed 30% of the uric acid employed in 12 hours and 100% in 24 hours, while *B. aerogenes* accomplished the destruction in 48 hours at 37°C. Although all the cultures were incubated for a period as long as 14 days they failed to destroy any portion of the substituted compounds. All growth ceased when the uric acid was completely utilized. In mixtures of uric acid and the substituted uric acids the organisms selectively utilized all the uric acid employed and left the methylated uric acids quantitatively unaffected. These results are comparable with the findings of Armstrong and Horton,[4] who showed that urease is capable of acting on urea itself but not on methyl urea, s-dimethyl urea, as dimethyl urea, ethyl urea or s-diethyl urea. In other words, the bacteria and the enzyme (urease) exercise a selective effect.

5548

A New Pathological Condition of Probable Dietetic Origin in Rats.

CASIMIR FUNK, SAUL CASPE AND HELEN CASPE.

From the Casa Biochemica, Rueil-Malmaison, (S & O), France.

It has recently been observed in this laboratory, that when young or mature rats, of both sexes, which have been on the laboratory stock diet (milk, white bread and mixed grains) are transferred to individual cages with screen bottoms and are fed on an artificial food mixture, consisting of 18% casein (A and B free, British Drug

[4] Armstrong, H. E., and Horton, E., *Proc. Royal Soc. of London*, 1912, **85**, 109.

Houses), 78% corn starch and 4% McCollum's salt mixture, they develop, in the majority of cases, within 8 to 10 days, peculiar tail symptoms. These symptoms start with a slight segmentation of the distal end of the tail, followed by a more accentuated constriction, reddening of the segment, sometimes bleeding, necrosis and falling off of the tail tip. As the experiment proceeds the segmentation and the process extends.

It has been thought first, that the symptoms present an analogy with the deficiency disease described by Burr and Burr.[1] However, the following reasons speak in favor of a new pathological entity. The disease observed by us develops much earlier and is neither prevented by a daily addition of 400 mg. lard, 2 drops of cod liver oil, nor by 2 drops of linoleic acid. Replacing the purified casein by ordinary commercial casein did not delay the symptoms.

FIG. 1.

Of the 44 rats used in the initial experiments, 56.8% showed severe and 22.6% slight symptoms. The remaining 20.6% were normal looking animals. Of the several substances tried out so far, 50 mg. of dried yeast per day did not prevent the onset of sickness. Our experience seems to indicate that the tail symptoms are a part of a general syndrom as the development of severe lesions coincides

[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345; 1930, **86**, 587,

usually with an arrest in growth. Under investigation, at present, is the prophylactic effect of more substantial yeast doses, as well as other products.*

5549

Variations in the Micronuclear Apparatus of Paramecium bursaria.

LORANDE LOSS WOODRUFF.

From the Osborn Zoological Laboratory, Yale University.

Studies have been made on the micronuclear apparatus of a race of *Paramecium bursaria* in pedigreed culture for more than 6 years, with the following chief results.

During this period marked variations have occurred in the micronuclear number. Originally the animals were bimicronucleate, but later they assumed the unimicronucleate condition characteristic of the species, and finally became amicronucleate.

Since throughout the life of the culture there have been no marked variations in the vitality of the animals, whatever function the micronuclear apparatus plays in the somatic life of the race is not obviously influenced by profound changes in the volume and distribution of the micronuclear material.

Cytological investigations have revealed no evidence of endomixis or conjugation.

The viability of amicronucleate animals, without the power to undergo endomixis or conjugation, further supports the indentification of the macronucleus and micronucleus as a segregation of somatic and generative elements into discrete bodies within the cell.

* As the paper goes to press, we noticed a paper by Hume and Smith (*Biol. J.*, 1931, **25**, 300) dealing with the same phenomenon. While we differ somewhat in the interpretation of the facts, we agree on the main results.

5550

Effect of Silica on the Growth of the Tubercle Bacillus.*

R. M. PRICE. (Introduced by Oskar Klotz.)

From the Laboratories of Pathology and Bacteriology, University of Toronto.

In silicosis, silica in some form or in some way seems to increase the pathogenic effects of the tubercle bacillus. It therefore suggested itself that silica might have a stimulating effect on the growth of the tubercle bacillus *in vitro*. Accordingly, I carried out a number of cultural experiments, by adding 1 cc. of sodium silicate or silicic acid, each standardized to contain 1 mg. of the silica used in the preparation per cubic centimeter, to every 100 cc. of Dorset's egg medium, modified by replacing the distilled water by a beef infusion free from added peptone, or sodium chloride, and adjusted to a pH of 7.6.

The results obtained in these preliminary studies have been consistently uniform, and indicated a definite stimulating effect of the added substances, even though the estimated quantity of silica is 0.001 mg. per cc. of medium. This is shown by a lessening of the latent period, and a greater luxuriance of growth. The stimulating effect appeared to be continuous throughout the period of growth. I was thus able to produce readily visible colonies in initial cultures from guinea pigs, and directly from human sources, such as pus, urine and ascitic fluid, in a much shorter time than with other media. In one instance, a primary culture was isolated from ascitic fluid on sodium silicate medium, whereas we failed to obtain any growth on the control tubes of our Dorset's egg medium, with or without glycerine.

Colonies became visible in about 6 days, growth continued rapidly, and could be considered luxuriant within 3 weeks. These observations now cover 20 primary cultures, 17 of which were isolated through guinea pig inoculation, and the remaining 3 directly from human materials.

After I had been successful in demonstrating this stimulating effect on the tubercle bacillus in 6 or 8 primary isolations, I was interested to learn whether this suggested relationship had not previously been tested out. The only reference to such a study was in a verbal communication from Dr. G. J. Cunningham, Director of the Bureau of Industrial Research of the Public Health Laboratories

* This research was carried out under a grant from the National Research Council of Canada.

of Ontario, who attended the Silicosis Conference in Johannesburg, South Africa, in the summer of 1930, and who informed me that Dr. Kettle in a paper read at the conference stated that by replacing the diluent in the egg medium by silicic acid he observed a lessening of the latent period of growth of the tubercle bacillus, but that the effect was merely temporary, the silicic acid having to be replaced at intervals. Since the proceedings have not yet reached me, I am unable to be sure of his actual findings.

5551

Factors Involved in Male Production by Crowded Moina macrocopa Mothers.

A. M. BANTA AND L. A. BROWN.

From the Carnegie Institution, Brown University, and George Washington University.

The writers[1, 2, 3] have extended the earlier observations of Grosvenor and Smith[4] and others on the association between crowding of *Moina macrocopa* mothers and the production of male offspring. We also noted an association between retardation in the time of production of the parthenogenetic young and the percentage of male young produced. Within limits the percentage of male young produced is roughly proportional both to the degree of crowding and to the amount of retardation in the time of their production. Excessive retardation, however, whether induced by crowding or by other treatments, is accompanied by a reduced percentage of male young. We have interpreted this retardation and this male production as due to the accumulation of the mothers' excretory products.

Stuart and Banta[5] have shown that quantity of bacteria available as food for Moina mothers appears, under certain appropriate experimental set-ups, to be the determining factor in sex control in this species. This finding might raise the question as to whether quantity of available food is the principal or sole influential factor involved in male causation in crowding or other experiments, by

[1] Banta and Brown, *Sci. Papers, 2nd Int. Cong. Eugenics*, 1923, **1**, 142.

[2] Banta and Brown, *Physiol. Zool.*, 1929, **2**, 80, 93, 302, 309; 1930, **3**, 48.

[3] Banta and Brown, *Proc. Nat. Acad. Sci.*, 1929, **15**, 71.

[4] Grosvenor and Smith, *Quart. J. Mic. Sci.*, 1913, **48**, 511.

[5] Stuart and Banta, *Anat. Rec.*, 1929, **44**, 210; *Physiol. Zool.*, 1931, **4**, 72.

Moina mothers. In certain experiments[6] involving aeration of mothers during the critical period[5] male production was reduced or eliminated, in which case quantity of food apparently cannot be considered the determining factor in sex control. But the results of crowding might seem readily interpretable on this basis.

This note records the results of experiments designed to differentiate between (1) the quantity of available food and (2) some other factor associated with crowding (presumably "the accumulation of excretory products") as factors in influencing male production.

Into a series of bottles, each containing 25 cc. of manure solution culture medium, were placed young sister Moina—1, 2, 4, 6, and 8 females per bottle. Several such experiments were conducted. The results were fairly consistent. No males were produced in the 1-mother bottle; occasionally some appeared in the 2-mother bottles; a large percentage of males in the 4-mother bottles; still more in the 6-mother bottles; and a considerable percentage of males were produced in the 8-mother bottles, though a smaller percentage than in the 6-mother bottles.

With the aid of Miss Maurita McPherson, bacterial counts were made in some of these series—both at the time they were set up and at the critical period for sex determination. These counts showed, as was anticipated, that the numbers of bacteria at the time of the second count had decreased. This decrease was slight in the control (blank) bottle; more in the 1-mother bottle; still more in the 2-mother bottle; and, in general, the numbers of bacteria had decreased more and more as the numbers of mothers in the bottles were increased. But of most interest was that the percentages of males increased progressively up to the stage of over-crowding. The percentages of males did not follow, even roughly, the numbers of bacteria available as food for the experimental mothers during the critical period for the determination of the sex of their young. The numbers of bacteria in the one-mother bottles at the critical period ranged from 1.2 to 25.4 millions per cc. No males occurred in any of these 1-mother bottles. In the 6-mother bottles, in which the maximum male production occurred, the numbers of bacteria ranged from 1.0 to 14.6 millions per cc.

Since there is a close and fairly consistent relationship between the amount of crowding of mothers and their male production, but no consistent relationship between numbers of bacteria available as food for mothers and the sex of their offspring, some change which

[6] Banta and Brown, *Physiol. Zool.*, 1929, **2**, 93.

the Cladocera mothers made in the culture medium, other than mere reduction of the available quantity of bacteria, appears to have been the determining factor in influencing the sex of the young produced.

5552

Oral Immunization of Humans Against Pneumococcus, Determined by the Increased Protective Antibody Content of Serum.

VICTOR ROSS.*

From the Bureau of Laboratories, Department of Health, New York City.

The writer reported[1] that protective antibodies against pneumococcus, type 1, could be found in the sera of rats fed either (1) infected pneumococcus tissue (2) the living organism, or (3) the acid killed organism. Not all sera of rats thus actively immunized were found to contain these transferable antibodies and in those that did, the amounts present neutralized from 10 to 100,000 fatal doses (in 0.20 cc.). Experiments performed on dogs demonstrated similar results. Neither agglutinins nor precipitins could be found in the blood of such orally immunized animals, a statement confirmed recently by Maeji[2] for a rabbit fed type 3 organisms. The absence of agglutinins and precipitins made it appear that a similar condition would possibly be found to exist in humans fed acid killed pneumococci, even if the subjects should be made actively immune by this procedure. Reliance would consequently have to be placed upon the detection of an increased concentration of protective antibodies as a means of determining immunity.

The experiments reported here were done between October, 1928, and October, 1930, and were briefly mentioned elsewhere.[3] The work was interrupted but is now being continued. The results obtained on 14 subjects are reported below.

The sedimented HCl killed organisms (type 1 throughout) were used directly after centrifugation or after desiccation and were administered generally on a fasting stomach. The quantities fed

* The author wishes to thank Mrs. Lawrance Harriman for kindly providing funds in aid of this work. He is also indebted to the Harriman Research Fund for a grant.

1 Ross, Victor, PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 273.

2 Maeji, Y., *Acta Scholae Univ. Imp.*, Kioto, 1929-30, **12**, 295.

3 Ross, Victor, *J. Exp. Med.*, 1930, **51**, 585.

are listed for each subject. Blood was examined before and after the feedings. 0.20 cc. of serum drawn into a syringe containing 0.20 cc. of the diluted culture (type 1) were injected intraperitoneally into mice. The table shows the dose of pneumococci (with and without serum) injected before and after ingestion of the organisms, and the result. In most cases 2 animals were injected with each dose. Only those subjects who showed either a definite increase or a probable one are listed in the table; those which were negative are described briefly in the text.

TABLE I.

Protective power of serum of humans before and after ingestion of pneumococci. S = survived. The number = the day on which the mouse died.

Dose cc.	V. R. before		After 2 days		After 11 mos.		J. P. before		After 2 days		After 8 mos.	
	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum
10^{-9}					S.S		S.3	S.S	S.S	S.S	S.S	S.S
10^{-8}	2	S	2	S	2.2	S.S	2.2	2.2	S.4†	S.S	2.2	2.2
10^{-7}	3	3	2	S	2.2	S.4*	2.2	2.3	2.2†	S.2	2.2	2.2
10^{-6}	2	3	2	S	2.2	S.S	2.2	2.2		S.S	2.2	2.2
10^{-5}		S		3		S.6		2.2		4.5		2.1
10^{-4}		2		3		2.4		2.2		S.2		2.2
10^{-3}	*Ill at time of test; lost 2 gm. in 4 days preceding test.							2.2		2.2		1.1
							†Pn. in heart					

Dose cc.	H. W. before		After 2 days		After 8 mos.		A. J. K. before		After 2 days		After 6 mos.	
	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum
10^{-9}	S.3	S.S	S.S	S.S	S.S	S.S	7○.S	1.S	S.S	S.S	S.3†	S.S
10^{-8}	2.2	2.2	S.4†	S.S	2.2	2.2	3.3	7○.S	2.2	S.S	3.2	S.5†
10^{-7}	2.2	2.2	2.2†	S.S	2.2	2.2	3.2	8○.S	2.2	S.S	2.6†	S.S
10^{-6}	2.2	2.3	2.2	2.2	2.2	2.2	2.2	6.3	3.2	S.S	3.3	7†.6†
10^{-5}		2.2		2.2		2.1		4.4		S.3○		3.3
10^{-4}								3.3		2.3		4.3
10^{-3}	†Pn. in heart		○Sterile					2.3		2.2		2.4

Dose cc.	D. P. before		1 day after		C. M. before		1 day after		M. L. before		1 day after	
	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum
10^{-9}	2†.S	S.S	S.S	S.S	S.2	S.S	S.S		S.S	S.S	S.7‡	S.S
10^{-8}	2.2†	S.2†	2.2	S.2†	S.2	S.S	S.2		2.2	2.2	S.3	S.S
10^{-7}	3.2	S.2	2.2	S.S	2.2	S.S	2.2	S.S	2.2	2.2	3.4	S.2
10^{-6}		2.2	2	2.S	2	S.S		S.S	2.2	2.3	3.2	3.3
10^{-5}		1.2		3.2		7†		S.S		2.2		2.2
10^{-4}		2.2		2.2				3.2				
10^{-3}								1.1				
	†Pn. in heart								‡Contaminant in heart.			

Results V. R., Before Oct. 23, 1928. Protection against 1 m.l.d. Since 10^{-9} cc. was not used for control mice the possibility that the serum protected against 10 m.l.d. is not excluded. The survival of

the 10^{-5} cc. serum mouse is probably not significant in view of the death of the 2 mice injected with 1/10 and 1/100 of this quantity, and is probably owing to an unusual resistance on the part of this animal. 2 *days after last feeding:* Protection against 100 m.l.d. Since 10^{-9} cc. was not used for control mice it is possibly equivalent to 1000 m.l.d. 11 *mos. after.* Definite survival of serum mice receiving 100 m.l.d. and of 1 of 2 injected with 1000 m.l.d. *Comment:* It appears that this subject developed antibodies which persisted for at least 11 months. There were neither agglutinins nor precipitins. Moist growth from 300 cc. on Oct. 31, Nov. 1, 3, 5, 7; 500 cc. on 8, 9, 10, 12, 14; 1L on 15, 16, 17, and 21.

J. O., Before Dec. 4, 1928. Samples of blood taken at 2 different times were examined. The first showed protection against at least 10,000 fatal doses; the second against a similar quantity. 3 *days after.* Survival against 10,000 m.l.d. 10 *mos. after.* Protection against approximately 10,000 m.l.d. *Comment:* No increase. There were neither agglutinins nor precipitins. Moist growth from 330 cc. on Dec. 19, 20, 21, 22, 24; 500 cc. on 26, 27, 28, 29, 30.

R. B. Before Feb. 1, 1929. One of 2 serum mice survived 1 m.l.d. 1 *day after:* Both serum mice survived 10^{-8} cc. which killed 1 of 2 controls. 8 *mos. after:* Both serum mice survived 1 m.l.d. *Comment:* Probably no antibody increase. Moist growth from 300 cc. on Feb. 7, 8, 9; 500 cc. on 11, 13, 16; 1L on 18, 19, 20.

A. M. Before Feb. 1, 1929. There is protection against 100 m.l.d. 1 *day after:* No change. 8 *mos. after:* The same. Dosage was same as for R. B.

J. P. Before Feb. 28, 1929. There is possibly protection against 1 m.l.d. 2 *days after:* One of 2 serum mice succumbed to 10^{-7} cc., although both receiving 10 times this dose survived. Since 1 of 2 serum mice survived 10^{-4} cc. we might balance it against the death of the 10^{-7} cc. mouse. This leaves the protection at probably 100 m.l.d. The deaths from 1000 m.l.d. are delayed. 8 *mos. after:* There is no protective power. *Comment:* Protection rose from 0 to 100 m.l.d. with a return to original value in 8 months or sooner. Moist growth from 300 cc. on Mar. 4, 6, 7, 8; 500 cc. on 8, 9, 11, 12, 13, 14; 1L on 15, 16, 17.

H. W. Before Feb. 28, 1929. There is possibly protection against 1 m.l.d. 10^{-9} cc. killed 1 of 2 controls and neither serum mouse. Control mice before and after are same as for J. P. 2 *days after:* Protection against 10 m.l.d. if 10^{-8} cc. which killed 1 of 2 controls is the m.l.d. 8 *mos. after:* No protection. *Comment:* There is very probably an increase in antibody content with a return to the

original value in 8 months or sooner. Dosage was same as for J. P.

E. L. A. Before Apr. 5, 1929. One of 2 serum mice survived 1 m.l.d. 2 *days after:* Both serum mice survived 1 m.l.d. (10^{-8} cc.) but one died of 10^{-9} cc. 6½ *mos. after:* 1 of 2 serum mice survived 1 m.l.d. *Comment:* No increase in protective power. Moist growth from 300 cc. on Apr. 18, 19, 20.

A. J. K. Before Apr. 5, 1929. Controls same as for E. L. A. before and after 2 days. Of the serum mice those receiving 1 and 10 m.l.d. can be said to have survived since the 2 mice which died a delayed death of 10^{-8} and 10^{-7} cc. contained no pneumococcus at autopsy. The serum mouse which died of 10^{-9} cc. must also be counted as a survivor since death in 1 day from this dose is unlikely. 2 *days after:* Protection against 1000 m.l.d. (10^{-5} cc.). One of 2 serum mice dying of 10^{-5} cc. was sterile. 6 *mos. after:* 1 of 2 controls died of 10^{-9} cc. If we disregard this death and the delayed death of 1 of 2 serum mice which died of 10^{-8} cc. we find a return to the initial value of 10 fatal doses. *Comment:* It appears that the protective power of this individual increased decidedly 2 days after the last of 3 successive daily feedings and returned to the original value after 6 months or sooner. Dosage same as for E. L. A.

D. P. Before Sept. 9, 1930. One of 2 controls died of 10^{-9} cc. Among the serum mice 1 of 2 survived 1 m.l.d. and 1 of 2, 10 m.l.d., if 10^{-8} cc. is taken as the m.l.d. or 10 times these amounts if 10^{-9} cc. is taken as the m.l.d. 1 *day after:* 1 of 2 serum mice survived 1 m.l.d., 2, 10 m.l.d., and 1, 100 m.l.d. *Comment:* There seems to have been some increase though it is not certain. Desiccated growth from 1 L. on Sept. 9, 400 cc., on 10th, 400 cc. on 11th.

H. L. Before Sept. 16, 1930. 1 of 2 serum mice survived 1 m.l.d. (10^{-9} cc.) and 10 m.l.d. respectively. 1 *day after:* Only 1 of 2 controls died of 10^{-8} cc., whereas both serum treated animals succumbed, although 1 after 4 days. *Comment:* It would seem as if a slight drop in protective power had taken place, but since this occurred in the neighborhood of 10^{-8} and 10^{-9} cc., doses where irregularities occasionally are found, the change was probably not real. Desiccated growth from 1 L. on Sept. 16, 400 cc., on 17 and 18.

C. M. Before Sept. 23, 1930. Protection against 100 m.l.d. if 10^{-8} cc. is taken as the m.l.d. for controls (only 1 of 2 died but 1 of 2 succumbed to 10^{-9} cc. also). If 10^{-9} cc. is the m.l.d. then the antibody content is 1000 m.l.d. 1 *day after:* Only 1 of 2 controls died of 10^{-8} cc. If this is the m.l.d. then there is protection against 1000 m.l.d. *Comment:* It seems as if an increase may have taken place

but this is not certain. Desiccated growth from 1 L. on Sept. 23, 400 cc. on 24, 25.

M. E. Before Sept. 23, 1930. Same controls as for C. M. before and after. Both serum mice succumbed to 10^{-8} cc. and one died of 10^{-9} cc., so there was no protection. 1 *day after:* No change in survival among serum mice. *Comment:* No change. Dosage same as for C. M.

S. F. Before Oct. 7, 1930. No protection. 1 *day after:* No protection. *Comment:* No change. Desiccated growth from 1 L. on Oct. 7; 400 cc. on 8, 9.

M. L. Before Oct. 7, 1930. No protection. 1 *day after:* 1 of 2 control mice injected with 10^{-8} cc. lived as against both serum mice. Both controls died of 10^{-7} cc. only 1 of 2 serum mice. *Comment:* There is possibly an increase but in view of the experience with H. L. one cannot be certain.

Summary and Discussion. There appears to be clear evidence for an increased antibody content in the sera of subjects V. R., J. P., and A. J. K. In the individuals H. W., D. P., and M. L. there also seems to have been some increase. In C. M. the result is in doubt. No change apparently took place in the remaining persons. Where the initial value for a serum is 100 to 10,000 m.l.d. (C. M., A. M., and J. O.) an increase equal to less than 900 to 90,000 m.l.d. cannot be detected by the method used. This may account for an apparently unaltered concentration of antibodies in some instances. It would seem, therefore, that human beings may develop protective antibodies following ingestion of dead pneumococci (type 1). The appearance of such antibodies in the sera of rats which have been actively immunized against the pneumococcus by similar treatment suggests that those persons who developed antibodies also became actively immune. The absence of a change in antibody content in the others may not necessarily mean the lack of an increased resistance to the organism, for the animal experiments also seem to indicate that in some individuals active immunity may be produced without such a change.

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Spermagglutination by Bacteria.

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The agglutination of spermatozoa under the influence of different factors, such as acids, alkalis, salts of the heavy metals, CO_2, dyes, substances derived from ova, etc., was observed by various authors. By studying the action of bacteria on spermatozoa *in vitro* we discovered a new agglutinating factor in some strains of *B. coli.*

For our experiments we used: 1. A saline suspension of spermatozoa of guinea pigs, rats, and rabbits (epididymis specimens), and human (condom specimens). 2. A broth culture of *B. coli* or a saline emulsion of the bacterial growth from an agar slant. The test was performed on a glass slide by mixing a drop of the sperm-suspension with a drop of the culture. In a control drop of sperm without bacteria, the single spermatozoa were evenly distributed in the fluid. But after mixing with the bacteria, the spermatozoa were almost instantaneously agglutinated and formed flocculi and clumps which could easily be seen with the naked eye. Microscopic examination with low and high dry power showed that the spermatozoa were clumped together into big net-like formations. At the same time most of the spermatozoa were immobilized. A few of them, although included in the clumped net-work, were still slightly motile, but after a few minutes they lost all signs of motility. Spermatozoa mixed with *B. coli* culture in a tube formed clumps which in a short time settled down, leaving the supernatant fluid clear and transparent The reaction was irreversible. Addition of acids or alkalis, or shaking did not disintegrate the clumps. The agglutination by bacteria did not depend on the pH of the medium. The clumping under the influence of bacteria took place in solutions of various pH covering the range from 3 to 10. Dead spermatozoa (heated at 56° for 1 hour or killed by chloroform) were susceptible to clumping by bacteria to the same degree as living ones.

The active principle is contained within the bodies of the bacteria. A broth culture passed through a Berkefeld filter does not affect the spermatozoa. On the other hand, the sediment of a broth culture after repeated washing with saline, still preserved its agglutinating power. Boiling the culture for 1 minute at 100°C. destroys the active principle, but heating at 60°C. for a prolonged time (24 hours and more) does not affect it at all, although the bac-

teria themselves are killed at this temperature. Subjecting the liquid bacterial culture to a vacuum at room temperature for one hour to expell gases does not interfere with the agglutinating power of the bacteria. Thus, the possible influence of CO_2 as the agglutinating agent can be excluded.

The age of the bacterial culture is of no significance. Those several months old are as active as one 24 hours old.

Addition of dyes (*e. g.*, trypaflavin solution 1:1000) also agglutinates the spermatozoa. But while this agglutination by dyes does not take place in the presence of normal serum,[1] the agglutination by bacteria does not suffer by the addition of serum.

Among many strains of *B. coli*, isolated on various occasions from feces and urine of patients, only few have been found to be active. Various other bacteria (strains of streptococci, staphylococci, pneumococci, gonococci, *B. typhi, B. paratyphi, B. proteus, B. pyocyaneus, B. subtilis, B. tuberculosis*) tested against spermatozoa gave negative results.

The sperm agglutination by bacteria is in all aspects analogous to the phenomena described by Lillie[2] as "sperma mass coagulation" and by Loeb[3] as "real sperm agglutination" and considered by them as detrimental to the fertilizing power.

Therefore the presence of spermagglutinating strains of bacteria in the vagina and in the cervix of the uterus should be taken into consideration as a potential factor of sterility.

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[1] Rosenthal, L., and Hornick, O., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 516.

[2] Lillie, F. R., *Biol. Bull.*, 1915, **28**, 19.

[3] Loeb, L., *J. Exp. Zool.*, 1914, **17**, 126; *Pflüger's Arch.*, 1904, **104**, 335.

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5554

Effect of Proteins on the Diffusion of Amino Acids Through Membranes.

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The concentration of amino acids in nucleated erythrocytes is known to be several times that of the surrounding plasma. The concentration in mammalian liver and muscle, as indicated by determinations on tungstic acid extracts, is 6 to 8 times that of the blood plasma.

As part of an inquiry into the factors that cause this inequality in distribution we have studied the diffusion of amino acids through tubular membranes of cellophane. The progress of dialysis was measured by amino nitrogen determinations on the inner and outer liquids by the manometric method of Van Slyke. The inner liquid consisted of a 1.5% solution of gold label gelatin. The amino acid was contained in the outer liquid in an initial concentration of 0.001 N. Glycine was generally employed. After 15 to 30 hours at a temperature of 20° the experiments were terminated. The outer fluid was analyzed directly, while the inner fluid was first rendered free of protein by treatment with phosphotungstic acid.

Over a wide range of H ion concentration, (pH 2.5-9.5) the equilibrium concentration of amino acid in the outer fluid was found to be over twice as great as that in the inner protein-containing solution. This inequality persisted, undiminished in magnitude, even in the isoelectric zone of the protein. The equilibrium, moreover, could be approached from the other end, that is by dissolving the amino acid in the inner fluid containing the dispersed protein. In neutral solutions aspartic acid and glutamic acid behaved like glycine in the establishment of a high concentration ratio when dialyzed against 1.5% gelatin. Dialyzed solutions of crystal-

line egg albumin (1.5%) and agar-agar (0.38%) affected the diffusion of glycine at pH 5 in similar fashion.

The persistence of this unequal distribution in isoelectric protein solutions demonstrates that the equilibrium is not of the Donnan type.

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Studies on the Combination of Manganese with Certain Amino Acids and Related Compounds.*

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The present work is a continuation of the studies which have been carried out in this laboratory on the combination of proteins, amino acids, and allied compounds with the inorganic elements.[1, 2, 3] This work has now been extended to include the manganous compounds.

The method employed consists in adding the substances to be tested to a solution of manganous chloride. The solution is adjusted to pH 9.25 by means of a borate buffer. The aqueous solution is shaken for 20 minutes with a solution of isonitrosoacetophenone dissolved in chloroform. The color of the chloroform solution is compared in a colorimeter with a chloroform solution obtained in a similar manner except that the test substance is omitted. If the substance tested forms a compound with manganese (*e. g.,* complex ions) such as to decrease the activity of the manganous ions in the aqueous phase, the color of the resulting chloroform solution will be less intense than the standard when both are shaken for a period of 20 minutes. If the manganous compound is dissociated to a greater extent no effect on the color will be observed.

The results show that oxalic, malonic, succinic, and glutaric acids have a decided influence in decreasing the color. The quantitative effect is greatest in the case of oxalic acid and least in the case of glutaric acid. The effect of the addition of aspartic or of glutamic acid is approximately the same as that of the corresponding nitrogen-free acids. The addition of alanine or of sodium chloride was

* Aided by a grant from the Chemical Foundation, Inc.

[1] Greenberg, D. M., and Schmidt, C. L. A., *J. Gen. Physiol.*, 1924, **7**, 287.

[2] Greenberg, D. M., and Schmidt, C. L. A., *J. Gen. Physiol.*, 1926, **8**, 271.

[3] Smythe, C. V., and Schmidt, C. L. A., *J. Biol. Chem.*, 1930, **88**, 241.

without effect on the color. The effect of glycine was slight and that of glycylglycine slightly more, but not so great as that of the dicarboxylic acids.

The present work is being continued.

5556

Allergy and Immunity in Coccidial Infections.

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Immunity to the several species of coccidia which occur in chickens has been thoroughly demonstrated by Tyzzer[1] and by Henry.[2] A similar host response has been shown to occur in rabbits (Bachman[3]) and in cats and dogs (Andrews[4]).

The purpose of this paper is to report the development of a similar immunity in guinea pigs infected with *Eimeria caviae,* together with the appearance of degenerate forms of the parasite in partially immune animals, observations on the production of hypersensitivity to the protein of the infecting organism, and a cutaneous hypersensitivity to *E. caviae* in this host.

The reinoculation of 35 previously infected guinea pigs has in all cases shown the presence of some immunity. In most cases the resistance has been sufficient to completely prevent the occurrence of clinical symptoms, which are invariably present in initial infections. While the mortality in guinea pigs upon infection with *E. caviae* for the first time has been found to be 40%, not one of the guinea pigs infected 2 or more times died from a typical coccidial infection.

A rather constant indication of the effect of previous infection was the altered prepatent period in cases of the second infection. While this period has been found to be exceedingly constant in all of the initial infections, a variation from 2 to 4 days from the normal 11½-day period in the appearance of oocysts was found in most cases. A more striking indication of the effect produced by the host upon the parasite as a result of the development of partial immunity is the occurrence of degenerate cysts. These occur only

[1] Tyzzer, E. E., *Am. J. Hyg.,* 1929, **10**, 1.

[2] Henry, D. P., *Univ. Calif. Publ. Zool.,* 1931, **36**, 157.

[3] Bachman, G. W., *Am. J. Hyg.,* 1930, **12**, 641.

[4] Andrews, J. M., *Am. J. Hyg.,* 1926, **6**, 784.

in the *later* stages of initial infections, whereas in instances of the second to the sixth infections thus far tested they occur in the *early* stages, either alone or associated with normal cysts.

In some instances, guinea pigs which were fed mature oocysts after recovering from a previous infection died within 2 to 10 days thereafter with symptoms and lesions differing in all respects from those found in coccidiosis as observed in this host. Death was usually sudden and preceded by symptoms typical of those described for delayed anaphylaxis in guinea pigs. At autopsy severe hemorrhage was found to have occurred in the lungs, and the intestines were noticeably hyperemic. Cultures of the various organs were in most cases sterile, and in those cases in which growth was obtained a miscellaneous group of the commoner non-pathogenic bacteria was found. Similar symptoms have been produced in animals sensitized by subcutaneous injections of oocysts and subsequent intraperitoneal inoculations of the shock dose.

Intradermal injections of oocysts of *E. caviae* into guinea pigs which had recovered from infection with this coccidium have clearly demonstrated a cutaneous hypersensitivity. This was manifested by the appearance in approximately 48 hours of an inflamed area surrounding the point of injection, swelling, induration, and somewhat later, by central necrosis. In the only animal which received an intradermal injection while suffering from an active infection, the response occurred within 2 hours after injection. An area 3 cm. in diameter surrounding the point of injection became bright red at the end of 18 hours. Twenty-four hours after the injection the animal died. Six normal animals injected at the same time failed to show any reaction other than a slight irritation at the point of inoculation, which disappeared in 24 to 48 hours.

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Effects of Carbon-Dioxide Inhalations on Intrapleural Pressure in Dogs.*

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In our studies on the effects of broncho-constricting drugs on in-

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trapleural pressure,[1] we noted that whereas a more positive intrapleural pressure on expiration developed following the administration of such drugs, it was by no means as great proportionally as the development of a more negative intrapleural pressure on inspiration. This suggested that mere broncho-constriction was not the only factor operating to produce the effects. Since resistance to the passage of air in and out of alveoli may be expected to increase gradually the CO_2 tension in the alveoli, and in the blood, we determined to study directly the effects of CO_2 inhalation on intrapleural pressure.

Dogs were lightly anesthetized with sodium amytal or with dial and urethane. The experimental technique employed has been previously described.[2] Inhalations of CO_2 concentrations of 10% in oxygen (22 experiments) are followed by a gradual development of more negative intrapleural pressure than exists normally. The effect is similar to, but more marked and rapid than that noted after the administration of such broncho-constricting drugs as pilocarpine or eserine. It is accompanied by an increase in mean thoracic girth. Respiration, in other words, proceeds with the chest in an inspiratory position. This is probably due to the initial stimulating effect of CO_2 on what Lumsden[3] postulated as the apneustic or inspiratory center in the medulla, and it probably is the cause of the more negative intrapleural pressure. Inhalations of concentrations of CO_2 higher than 10% in oxygen (16 experiments) lead to a quick change in intrapleural pressure, in which it not only becomes much more negative on inspiration but also much more positive on expiration. The latter effect is not as great proportionally as the development of more negative intrapleural pressure on inspiration. The average thoracic girth is increased as with lower concentrations of CO_2, but in addition there is a decrease in mean abdominal girth. This may be explained again on Lumsden's thesis that the expiratory center is stimulated only by relatively high concentrations of CO_2, although the chest remains in an inspiratory position. With the vagi cut (3 experiments), the inhalation of CO_2 in oxygen resulted chiefly in the development of a more positive intrapleural pressure, especially on expiration.

Our findings may explain in part the effects on intrapleural pressure we noted after the administration of broncho-constricting

[1] Brill, S., Prinzmetal, Myron, and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 617.

[2] Brill, S., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 518.

[3] Lumsden, T., *J. Physiol.*, 1923, **58**, 111.

drugs. There is direct evidence that resistance to breathing increases alveolar CO_2 concentration.[4] While it is often stated that CO_2 itself causes broncho-constriction, direct bronchoscopic observation has shown that CO_2 inhalations in man seem always to dilate the bronchi.[5] Our observations confirm and extend Lumsden's thesis that CO_2 first stimulates an inspiratory medullary center, and later, or with higher concentrations, an expiratory medullary center. We believe that any significant effect on intrapleural pressure in an intact body is mediated by central respiratory control in determining whether respiration shall continue with the chest in a relative inspiratory or in a relative expiratory position. The effects on intrapleural pressure of broncho-dilatation or broncho-constriction,

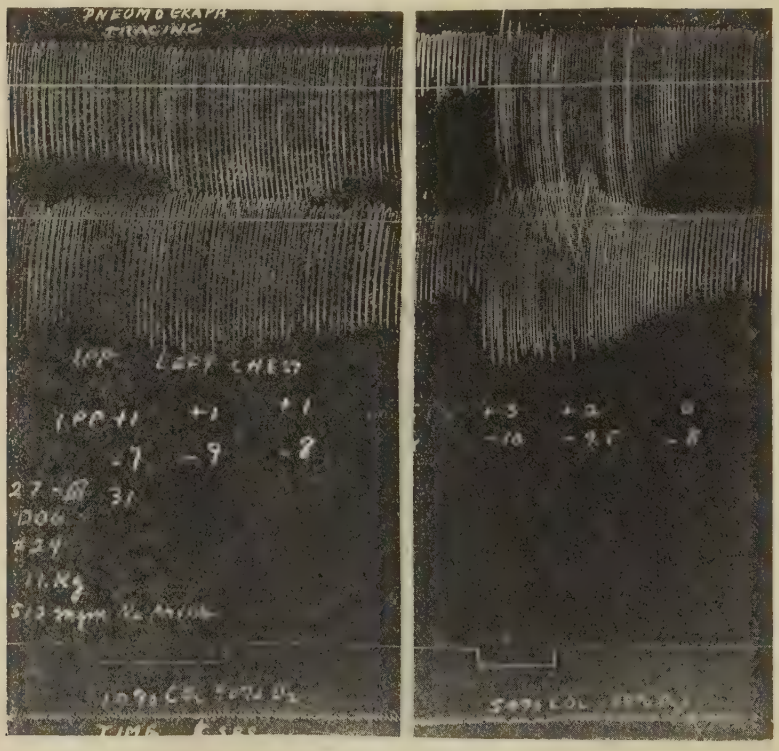

FIG. 1.

Kymographic record of effects of inhaling concentrations of 10% CO_2 and 50% CO_2 in oxygen respectively on intrapleural pressure and chest size as indicated by pneumograph in an 11 kilo dog lightly anesthetized with sodium amytal. Downstroke in pneumograph tracing records inspiration. Intrapleural pressure figures given in centimeters of water.

[4] Davies, H. W., Haldane, J. S., and Priestley, J. G., *J. Physiol.*, 1919, **53**, 60.

[5] Brunn, H., and Brill, S., *Ann. Surg.*, 1930, **92**, 801.

whether caused by drugs or otherwise, are probably governed by this factor. An explanation may thus be afforded for the emphysema noted in chronic asthmatics or after prolonged exposures to rarefied atmospheres. In the former case, the slight increase in alveolar and blood CO_2 tension due to broncho-constriction may act as the ever present stimulant to the inspiratory medullary center tending to fix the chest more and more in an inspiratory position. In the latter instance chronic oxygen want may be the continued stimulant.

TABLE I.

Effects of the inhalation of 10% CO_2 in oxygen on intrapleural pressure and thoracic girth in a 17 kilo dog lightly anesthetized with sodium amytal.

	Intrapleural Pressure in Cm. H_2O		Thoracic Girth	
	Inspiration	Expiration	Inspiration	Expiration
			cm.	cm.
Normal	—4.5	+0.5	54.6	54.2
CO_2 inhaled	—8.0	+0.5	55.3	54.2
CO_2 off	—6.5	+0.5	55.0	54.2

These considerations give further support to the use of CO_2 inhalations in the prophylaxis or treatment of post-operative atelectasis. Stimulation of an inspiratory medullary center by increasing the relative size of the thorax, and thus by making intrapleural pressure generally more negative, would tend to pull out a collapsed area of the lung. Since certain operative procedures, or the pre-operative use of atropine, may make intrapleural pressure more positive, and thus favor atelectasis,[6] CO_2 inhalations during or after operation are indicated to combat this tendency, in addition to its use as an adjunct or synergist to anesthesia.

[6] Brill, S., and Leake, C. D., *Am. J. Physiol.*, 1930, **93**, 636.

5558

Changes in Thoracic Size Following the Administration of Broncho-constricting or Broncho-dilating Drugs to Dogs.*

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Report has been made of the intrapleural pressure changes occurring after the administration of typical broncho-constricting or broncho-dilating drugs to dogs.[1] It has been suggested that these alterations in intrapleural pressure may be mediated by variations in thoracic girth accompanying the effects of these drugs on the respiratory mechanism, especially as a result of changes in alveolar and blood carbon dioxide tension following broncho-constriction or broncho-dilatation. The experiments here reported were undertaken to determine directly whether alteration in thoracic size actually occurs after the administration of broncho-constricting or broncho-dilating drugs.

The technique used for intrapleural pressure determination was the same as that previously described. In addition we measured directly the circumference of the thorax about 5 cm. above the xiphoid process, and we obtained records of respiratory movement by kymograph and pneumograph. After the subcutaneous injection of solutions of such broncho-constricting drugs as pilocarpine nitrate (2 experiments) and eserine salicylate (4 experiments), we found uniformly an *increase* in average thoracic girth accompany-

TABLE I.

Effect on intrapleural pressure and thoracic girth of the subcutaneous injection of 6 mgm. atropine sulphate in a 21 kilo dog anesthetized with sodium amytal.

	Intrapleural Pressure in Cm. H_2O		Thoracic Girth	
	Inspiration	Expiration	Inspiration	Expiration
			cm.	cm.
Normal	—2.8	0.0	25.2	25.1
5 min. after drug	—2.1	+3.0	25.0	24.8
10 " " "	—2.0	+7.0	24.8	24.5
15 " " "	—2.0	+8.0	24.6	24.4

* Supported in part by the J. J. and Nettie Mack and the Purington Research Funds.

[1] Brill, S., Prinzmetal, M., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 518; 1931, **28**, 617; 1931, **28**, 832.

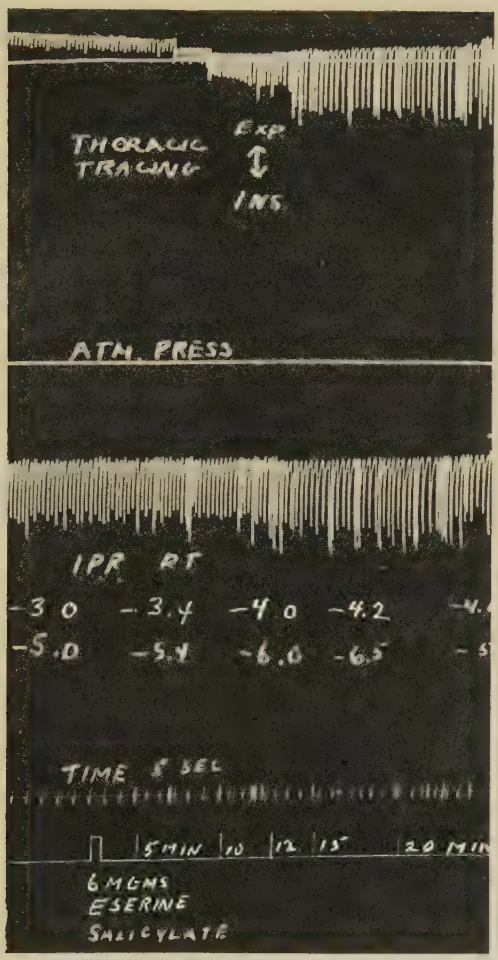

FIG. 1.

Effects of the injection of eserine, a typical broncho-constricting drug, on intrapleural pressure and thoracic girth as determined by pneumograph in a 12 kilo dog lightly anesthetized with sodium amytal. Intrapleural pressure figures are indicated in centimeters of water.

ing a more negative intrapleural pressure (Fig. 1). This corresponds to the change in thoracic size accompanying the more negative intrapleural pressure resulting from the inhalation of 10% CO_2 in oxygen. We found a *decrease* in mean thoracic girth (Table I) accompanying a more positive intrapleural pressure after the administration of such broncho-dilating drugs as atropine sulphate (3 experiments) and epinephrine hydrochloride (1 experiment).

We may postulate that the effects of broncho-constriction or

broncho-dilatation on intrapleural pressure are mediated by a sequence of events, causally related, as follows:

Broncho-constriction	Broncho-dilatation
↓	↓
Increased alveolar and blood CO_2 tension	Diminished alveolar and blood CO_2 tension
↓	↓
Stimulation of inspiratory medullary center	Relative depression of inspiratory medullary center
↓	↓
Increased inspiratory muscular activity	Diminished inspiratory muscular activity
↓	↓
Increased average thoracic size	Reduced average thoracic girth
↓	↓
More negative intrapleural pressure	More positive intrapleural pressure

While our experimental evidence presented in this and previous reports does not prove that a causal relationship exists between the sequence of events as listed in these schema, we feel that it is sufficient to establish a working theory in the absence of any significant evidence otherwise.

5559

Iodochloroxyquinoline (Vioform, N.N.R.) as an Amebacide in Macaques.*

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(Introduced by C. D. Leake.)

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In a previous study of a series of halogenated oxyquinoline derivatives, iodochloroxyquinoline (vioform, N.N.R.) was shown to be an effective balanticide in guinea pigs, with relatively low toxicity on repeated administration to monkeys.[1] The soluble hydrochloride of the basic ether of this compound was found to kill amoebae *in vitro* in high dilutions. On this basis we began to investigate the

* Part of an extended cooperative study of the chemotherapy of amebiasis, supported in part by the Ciba Co., Inc., New York City, and Eli Lilly and Co., Indianapolis.

[1] Anderson, H. H., David, N. A., and Koch, D. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 484.

amebacidal effect of vioform, N.N.R., in macaques naturally infested with *Entamoeba histolytica.* Similar studies were made by Kessel[2] with sodium iodoxyquinoline sulphonate ("yatren" or chinofon, N.N.R.) in monkeys. Doses of from one-half to 2 gm. were given daily, by this worker, to 4 macaques for 4 weeks. Two animals died in the second week of treatment when given 2 gm. of this drug daily. The 2 surviving monkeys received one-half gm. daily for 4 weeks and their stools remained negative for *Entamoeba histolytica* for a 3 months period.[3]

Dobell[4] has demonstrated the effectiveness of emetine bismuth iodide in monkey amebiasis. Twelve mgm. per kilo orally given daily for about a week cleared 4 of 5 macaques of *Entamoeba histolytica.* Four of these animals, however, exhibited signs of emetine toxicity during the course of this rather vigorous treatment. This fact was not sufficiently emphasized by Dobell but should be remembered before attempting to apply these results to human therapy. His suggestion "that macaques . . . . be utilized . . . . in place of men in future chemotherapeutic experiments" is certainly timely.

Eight monkeys, naturally infested with *Entamoeba histolytica* and various other intestinal parasites, were used in this study. In an effort to reduce reinfection to a minimum, animals were kept singly or in pairs in wire cages, which were fitted with false bottoms of 1 inch heavy mesh wire, so that droppings could fall through to the floor below. The cages were scoured daily and every attempt was made to prevent contamination of the food and water given the animals. They had a varied diet of high vitamine content rich in carbohydrate. The temperature of the room in which the monkeys were kept was between 18° and 22°C. Fresh stools were examined daily or every second day, during both a control period of observation and the course of treatment, and for 3 months afterwards. Fixed wet iron haemotoxylin preparations were examined routinely, and the stools also were cultured for amoeba and for organisms of the colon typhoid group. Three naturally infested but untreated monkeys were kept under identical conditions. Their stools remained positive for amoebae during the period in which the treated animals were studied.

Three monkeys *(Macacus rhesus)* were given powdered vioform, N.N.R., in gelatine capsules by mouth in divided doses over a 6 weeks' period. A total dosage of 900 to 1200 mgm. per kilo ren-

[2] Kessel, J. F., *Univ. Calif. Publ. in Zool.*, 1928, **31**, 275.

[3] Kessel, J. F., personal communication.

[4] Dobell, C., and Bishop, A., *Parasitology*, 1929, **21**, 446.

dered the stools negative for *Entamoeba histolytica* during the time of treatment, and for a three months' observation period afterwards. Other intestinal parasites were not consistently affected by the drug except that one animal was cleared of *Balantidium coli* and *Strongyloides stercorales* infestation. The remaining 5 monkeys were *Macacus cynomolgus;* 2 receiving 1 gm. per kilo of vioform, N.N.R., in 3 weeks time, 2 were given the same amount in

TABLE I.—Summary of Data on Vioform Treated Macaques.

Animal Species Sex	Total dosage mgrm./kg.	Period of treatment weeks	Weight in gm.	Stool Examination									Character of stools	General condition after treatment
				E. histolytica	*E. coli*	*E. nana*	*Iod. butschlii*	Chilomastix	Giardia	Trichomonas	Oesophagostoma	Balantidium coli	Strongyloides	
M-342	1200	6	a 2850	+	++	+	+	++	o	o	+	o	o	formed	excellent
M. rhesus			b 3250	o	+	o	o	+	o	o	o	o	o	,,	
Female															
M-335	900	6	a 2550	+	++	o	o	++	o	o	o	o	o	,,	,,
M. rhesus			b 2675	o	++	o	o	++	o	o	o	o	o	,,	
Female															
M-104	1000	6	a 2450	+	++	++	+	++	o	++	++	+	+	,,	,,
M. rhesus			b 3000	o	+	+	o	++	o	++	+	o	o	,,	
Male															
J-201	1000	3	a 1300	+[c]	++	++	o	+	o	o	+	o	o	,,	,,
M. cynomolgus			b 1400	o	+	+	o	o	o	o	o	o	o	,,	
Male															
J-202	1200	6	a 1250	+*	+	+	+	+	o	+	+	+	++	diarrhea	good
M. cynomolgus			b 1275	++	o	o	++	o	o	++	o	o	+	formed	
Male															
J-203	1000	4	a 1650	+	o	o	+	+	o	+	o	o	o	,,	excellent
M. cynomolgus			b 1700	o	o	o	+	+	o	o	o	o	o	,,	
Female															
J-204	1000	3	a 1500	+	++	++	+	+	++	++	o	o	o	dysentery	,,
M. cynomolgus			b 1550	o	+	+	o	o	+	+	o	o	o	formed	
Female															
J-205	1000	4	a 1200	+	++	+	o	++	+	++	+	++	o	,,	,,
M. cynomolgus			b 1250	o	+	o	o	+	o	+	o	+	o	,,	
Male															

a = before treatment. b = 3 months after cessation of treatment. c = Charcot-Leyden crystals.
+ = positive o = negative. * = motile forms.

divided doses over a 4-week period, while the fifth monkey, J-202, received 1200 mgm. per kilo in 6 weeks. All became and remained free of *Entamoeba histolytica* for the full period of study except J-202. This macaque was vomiting, suffered from dysentery, and was very emaciated before treatment, and showed motile amoebae in the stools. While his general condition improved markedly during observation we were unable completely to eradicate his amoebae. *Strongyloides stercoralis* was not affected by the drug in this monkey, but *Balantidium coli* was. In J-205, however, the *Balantidium coli* infestation was not cleared. Table I summarizes our results.

Summary. Seven of 8 macaques, naturally infested with *Entamoeba histolytica* have now remained free of these parasites for 3 months after receiving orally 900 to 1200 mgm. per kilo of iodochloroxyquinoline (vioform N.N.R.) given in divided doses over 3 to 6 weeks. The stools of 2 of 3 monkeys infested with *Balantidium coli* have been negative during this follow-up period. Of the 2 animals harboring *Strongyloides stercorales,* one was cleared of this infestation. We believe that important factors in our results were the hygienic conditions maintained and the precautions taken to prevent reinfection in animals under treatment. All treated animals gained weight, developed normally, and showed marked improvement in general physical condition following our therapeutic regime. No evidence of drug toxicity was noted in any animal.

5560

The Vaginal Smear of the Ewe.

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As an introduction to the study of the physiology of reproduction in sheep we have investigated the vaginal smear of 15 ewes daily for variable periods of time, 6 of them for 11 months. Three of these have been followed through pregnancy and lactation. Data have also been accumulated regarding the lengths of the breeding periods, oestrous cycles and the length of oestrum. Oestrum was first evidenced about the first of September and 6 of the sheep that were maintained went into anoestrum about the first of March, a breeding period of about 6 months. Our data in regard to the

length of the cycle confirm that recently given by Allen *et al.*[1] The length of the cycle is slightly less than 17 days on the average with extremes ranging between 16 and 23 days. There was only one instance which fell outside of the 16-18 day period.

There are well defined changes in the character of the vaginal smear depending upon the time of the oestrous cycle. In general it may be said that these changes are more apparent macroscopically than microscopically. On the first day of heat, and in rare instances on the second, the smear consists of transparent mucus in which one finds floating cell flocculi. The cells present are polymorphonuclear leucocytes, cornified cells, and small epithelial cells. On the second day of oestrum or on the first day of metoestrum the smear becomes dry and has a cheesy appearance. Cornified cells and small epithelial cells are present. Large numbers of leucocytes are usually present for about a day on or between the second to seventh day of metoestrum. Cornified cells are never completely absent and in fact, one often encounters smears which appear similar to early metoestral smears in dioestrum. Thus it is impossible to differentiate metoestrum and dioestrum clearly by means of the vaginal smear. The vaginal lumens of 2 animals autopsied on the seventh day after oestrum contained a copious quantity of cheesy cellular material. Thus it would appear that desquamated cells are not rapidly flushed from the vaginal lumen in this species.

On the day before the impending oestrum the smear is usually scanty in amount. The cell types are similar to those occurring on the first day of oestrum. Therefore the conspicuous features of the smear are as follows: Transparent mucus upon which one finds floating cell flocculi on the first day of oestrum; a dry cheesy mass consisting of cornified cells and small epithelial cells in early metoestrum; a mild leucocytic wave; and the scanty smear consisting of leucocytes, cornified cells, and small epithelial cells in late dioestrum.

During pregnancy the smear is very scanty and exhibits very little variation from day to day. Small epithelial cells and a few leucocytes compose the smear.

We have designated as anoestrum the long interval, about 6 months, during which oestrum is not expressed. Our data, however, do not support the view that the reproductive organs of the ewe assume a state of true rest throughout this period. In only the middle portion of anoestrum does the smear assume a rather monotonous character. In the early and late portions one finds changes

[1] Allen, Edgar, McKenzie, F. F., Kennedy, J. W., and Beare, W. K., *Anat. Rec.*, **48**, supplement, 9.

in the character of the vaginal smear comparable to those found in the active breeding period. In some instances we have encountered smears resembling so closely those found during oestrum that only after tests with 2 vigorous males were we convinced that the animal was not in true heat. Two or more of these inhibited cycles were usually recognized in early and late anoestrum. A similar observation was made upon 2 ewes during the third month of their lactation periods. In one instance true oestrum occurred. Histological study of the reproductive organs of the ewe at various times of anoestrum and lactation should give further data on this point.

5561

Ineffectiveness of Prolan in Hypophysectomized Animals.*

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A complete hypophysectomy with cautery at the base of the brain was done by Frederick L. Reichert on a female collie (hybrid) puppy 7 weeks of age from a litter where fortunately we had a sister control. Every 2 weeks, weighings and radiographs of the leg and skull were taken. After 2 such periods it was evident that the hypophysectomy was complete. Six months after the operation daily administration of Prolan was begun and continued for 53 days. An average of 30 cc. was given daily. At this juncture an ovary and one uterine horn were removed and sectioned. The naked eye impression of infantilism in the genitalia was verified. The animal was allowed to rest for 2 weeks. For a month thereafter about 15 cc. daily of a more potent preparation of Prolan was administered. This was proven to contain 80 to 100 rat units per cc. Death from a pneumonia at this juncture terminated the experiment but examination of the remaining ovary and uterine horn showed essentially the same conditions as were previously found, *i. e.*, a persisting true infantilism.

* Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, and from the Rockefeller Foundation. These funds have been generously augmented by the Board of Research and the College of Agriculture of the University of California.

Two other puppies were totally hypophysectomized at 6 weeks of age and subsequently similarly treated with Prolan—one of them a year after the operation and hence open to the objection that an irretrievable recession and fibrosis of the ovaries had resulted before treatment. The second puppy was hence treated 2 months after the operation but in spite of prolonged high dosage with Prolan the results were completely negative as in the first case reported.

A female rat 15 weeks of age and weighing 172 gm. was hypophysectomized by Richard Pencharz, on November 4, 1930, and its body weight and dimensions followed for several weeks to prove the completeness of the removal, which was established on January 14, 1931, when a laparotomy was done. One ovary was removed and its infantile character established by section. Four days later, the daily administration of 100 units (1 cc.) of Prolan was begun and continued for a week without the slightest effect on the sexual sphere (behavior, character of vulval lips, and vaginal smear).

After a pause of 20 days, the experiment was repeated for a duration of 10 days with similar non-effect confirmed by examination of the internal organs. The experience harmonizes completely with that with the hypophysectomized dogs.

The experiments, especially the very prolonged ones with dogs, both from the standpoint of duration and height of dosage with Prolan, of proven potency, appear to amply establish the incapacity of this substance to reestablish the normal development and function of the reproductive system after hypophysectomy. In this ineffectiveness, it is in contrast with the successful effect of anterior hypophyseal substance itself.†

† After our experiments were concluded, the publication of Hill and Parkes appeared with a different animal material and a different test. These investigators secured results which we consider analogous to our own. They reported the practical failure of Prolan to produce ovulation in decerebrated rabbits. The deductions to be drawn from both kinds of experiments will be clear in the second paper of this series.

5562

Relation of Prolan to the Anterior Hypophyseal Hormones.*

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In our preceding paper we have shown the incapacity of the hormone found in the urine of pregnant women (Prolan) to affect sexual development and function in hypophysectomized animals. It is now well established that Prolan is also for some reason limited in its effect on the gonad development which can be produced within 100 hours in immature normal animals, as measured in terms of ovary weight.

We can, for example, state that no matter how frequently or how much Prolan is administered the average weight of the ovaries produced in 26-day-old rats at the close of 4 days of treatment is about 60-70 mg. and the maximum in several hundred of our experiments was 102 mg. We, and other investigators, have pointed out that in contrast with this the implantation of anterior hypophyseal tissue is very markedly more effective in the ovary weights produced in young rat hosts, and we have shown that increasing "doses" of the implants also give increasing weights of the young ovaries.

As will be detailed in another communication, similar very marked effects on the immature gonads can now be secured by certain fractions out of extracts of anterior hypophyseal tissue. Here also, in contrast to Prolan, increasing doses give increasing effects on the youthful gonads. With such preparations, we have, for instance, in 100 hours produced ovaries weighing 190 mg.

We searched for a point of view which would harmonize these 3 experiences. That Prolan is totally ineffective in hypophysectomized animals and that it is only partially effective in young animals when compared with cases to which actual hypophyseal substances were administered seemed to us best explained by regarding Prolan only as an activator to a substance in the hypophysis itself which we may call a pro-hormone, a substance totally absent, of course, in hypophysectomized animals and small in amount in very young normal animals. This idea seemed capable of simple test by the simul-

* Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, and from the Rockefeller Foundation. These funds have been generously augmented by the Board of Research and the College of Agriculture of this University.

taneous administration of Prolan and hypophyseal hormones, the effect of each of which alone was previously known. The following table and description of a single experiment will illustrate our results:

TABLE I.

Effects on the youthful ovary of Prolan and hypophyseal hormone given separately and in combination.

	Total Dosage During 3 Days mg. dry weight	Average Weight of Ovaries after 100 hours mg.
Prolan	54.2	62 (6-12 corpora lutea, a few large follicles)
Hypophyseal hormone	65.0	21.3 (1-6 small corpora, otherwise undeveloped)
Prolan combined with hypophyseal hormone	119.2	156.3 (Innumerable corpora and many large follicles)

We purposely chose a small dose level of an hypophyseal extract containing both growth and gonad-stimulating properties which, when given alone, either failed or barely provoked sexual maturity at the close of the 100-hour period in young animals. We selected a Prolan preparation which, when administered alone produced the normal maximal effect of Prolan preparations, that is, ovaries weighing about 60 mg. at the close of the test period. As the table shows, the mixture of these particular Prolan and hypophyseal extracts each given in the same dosage and same volume as when administered alone now produced ovaries averaging 156.3 mg.

We have confirmed this activation of hypophyseal extracts by Prolan in several hundred individual experiments. That Prolan thus activates a specific substance in hypophyseal extracts is shown by its failure to activate when the hypophyseal extracts have been heated to 100°C. for 10 minutes or to 70°C. for 30 minutes, in which procedure no noticeable changes in any of the physical properties of the extract could be detected.

Most surprisingly, it is not the gonad-stimulating hormone of the hypophyseal extracts which is activated by Prolan, for solutions rich in this hormone and almost devoid of the growth principle can not be activated, whereas growth hormone preparations free from gonad-stimulating properties are typically activated.

From preliminary quantitative work it would appear that the activation effect is dependent on the concentration of both constituents, the Prolan and the hypophyseal component.

Preliminary hypotheses must include at least 2 explanations of the

phenomena we have presented. The activator either converts an inactive pro-hormone in the hypophysis to an active form or else is able actually to convert the growth hormone into the gonad-stimulating one. It is attractive to strike an analogy here with the conditions found in the interaction of certain ferments and their co-ferments, for example the interaction of trypsinogen and enterokinase to produce tryptase, or again the cooperative action of the lactic-acid-forming enzyme with its co-ferment.

5563

Ionic Migration of Bismuth in Different Bismuth Products Under Different Conditions.

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Cerebral and spinal fluid penetration has not been established with certainty for all products of bismuth, and the efficacy of bismuth in cerebrospinal syphilis has been asserted and denied. It is conceivable that the variations here might be related to the amphoteric character of the metal which would vary with the product used. That is, penetration and activity might be favored by anionic bismuth, in accordance with the well-known composition of cerebrospinal fluid in which anions exist in relatively higher concentration than cations, and with the well-known appearance of the anions bromide, iodide and salicylate in this fluid. Correlated with these phenomena are the demonstrated penetration of the anionic bismuth compounds, sodium bismuthate and sodium iodobismuthite, and the negative or undependable penetration of the cationic bismuth products, bismuth metal, bismuth salicylate and potassium bismuth tartrate, previously reported from this laboratory.[1] Since the ionic character of bismuth in iodobismuthite only had been established in our previous work, the other products having been accepted on theoretical grounds, it appeared desirable to establish experimentally the ionic character of bismuth in various products under different conditions. Some of these products are claimed to penetrate the brain, and have been accepted for *New and Non-official Remedies* since our original work.

[1] Hanzlik, Mehrtens, Gurchot and Johnson, *J. Am. Med. Assn.*, 1931, in press; Gurchot, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 509.

Six different products of bismuth were tried, in aqueous solution (really after hydrolysis of some products), in special media such as 25% sucrose (bismuth sodium tartrate), 6% dextrose (bismuth metal) and ethylene glycol and alcohol (sodium iodobismuthite) and in dialyzed and natural horse serum. The bismuth-mixture was introduced into a convenient glass U-tube of 10 cc. capacity up to the level of the stop-cocks, one in each arm. Both cathode and anode chambers were filled with a non-conducting fluid, *i. e.,* distilled water, one of the special media mentioned above, or serum. Platinum electrodes and a current of 110 volts were used, but only from 10 to 45 millivolts passed through the bismuth-mixtures. Generally the current was passed through for 5 hours, but occasionally 3 hours and 26 hours. Incubations at 37.5°C. for 24 hours were made with the highly insoluble bismuth metal, bismuth salicylate and bismuthate in serum, in addition to the use of fresh serum-mixtures of these products. Saturated solution of hydrogen sulphide in water was added to each chamber and the presence of bismuth recognized by a black precipitate; in the case of iodobismuthite also by the presence of the colored ion which was photographed in color. At least 3 experiments were made with each product and condition; the results agreed invariably. The following tabulation gives a summary of the results obtained and a correlation with cerebral penetration as demonstrated by us or as claimed by others.

It is seen that the ionic character of the bismuth varied with the compound and with the conditions, thus indicating the complex behavior of bismuth. In bismuth metal, bismuth was found to be electropositive, as might be expected, and this was not changed in standing and incubated serum. Bismuth salicylate yielded a small amount of electropositive bismuth in incubated serum only, but the white aggregates moved to the cathode in both water and serum. These products are poorly absorbed and did not demonstrably, or at least dependably, penetrate the brain. In sodium bismuthate, the bismuth is obviously electronegative, but the product is so insoluble that, even after incubation in serum, bismuth ions were not demonstrated although yellowish aggregates moved to the anode (electronegative); bismuth was demonstrated in rabbit brains.[1] In sodium iodobismuthite, the bismuth was electronegative and combined with iodine, since the complex colored ion migrated without decomposition, in both aqueous media and serum: this bismuth has been demonstrated in animal brains and human cerebrospinal fluids[1] and its absorption is good. Bismuth sodium tartrate gave interesting results, since it yielded either electronegative or electropositive

TABLE I.
Electrical charges carried by bismuth in different bismuth products and cerebral penetration.*

Product	In Aqueous Medium	In Serum	Cerebral and Spinal Fluid Penetration
Bismuth metal (Bi)	+ = dextrosesol. + = water	none = fresh + = incubated + = stood 22 hr.	undependable or not demonstrable
Bismuth salicylate ($BiOC_7H_5O_3$)	None = water (+ = aggregates)	none = fresh + = incubated (+ = aggregates)	undependable or not demonstrable
Sodium bismuthate ($NaBiO_3$)	None = water (— = aggregates)	none = fresh none = incub. (— = aggregates)	demonstrated
Sodium iodobismuthite (Na_2BiI_5)	— = water — = ethylene glycol	—	,,
Sodium bismuth tartrate ($Na(BiO)_4Tart$) †	+ = sucrose sol. + & — = water	—	claimed
Sodium bismuth thioglycollate ($Bi(SCH_2CO_2Na)_3$) ‡	— = water	—	,,

* The plus sign (+) means electropositive (cation), and the negative sign (—), electronegative (anion).
† Product marketed by Searle and Co., Chicago.
‡ Product marketed by Parke, Davis and Co., as Thiobismol.

bismuth, or both. In 25% sucrose solution, containing a trace of NaOH, the product gave electropositive bismuth only, but after hydrolysis of this solution by direct treatment with water, there were electronegative bismuth and electropositive bismuth, in 1 experiment the ratio being about 1:4, respectively. In serum, the bismuth sodium tartrate gave electronegative bismuth only; this product was found to penetrate the brain of animals, but not cerebrospinal fluid in man; absorption was good. However, the literature on the tartrates of bismuth is contradictory. The bismuth of bismuth sodium citrate ($Na(BiO_2)Ci$), which is chemically analogous to bismuth sodium tartrate, is claimed to be anionic[2] and cationic.[3] Both claims may be correct, for this compound may contain either electropositive or electronegative bismuth, or both, according to the treatment it may be given. Sodium bismuth thioglycollate, in both water and serum, yielded electronegative bismuth only, the quantity being comparatively small; this bismuth is claimed to penetrate the brain.[4] It is possible that, in serum, some of the

[2] Oettingen, Ishikawa and Sollmann, *J. Am. Pharm. Assn.*, 1928, **17**, 124.
[3] Morton, *Quar. J. Pharm. Pharmacol.*, 1930, **3**, 561.
[4] Gruhzit and Sultzaberger, *Am. J. Syphilis*, 1927, **11**, 103.

products formed bismuth complexes. Changes in chemical reaction might affect the results, but were not considered, the products being used as in therapeutics.

Conclusion. The amphoteric character of bismuth in 6 different products used in the treatment of syphilis, and variations in the behavior of the metal under different conditions, were demonstrated. These properties are believed to be of significance for the pharmacological and clinical actions of bismuth, such as absorption, toxicity, cerebral penetration, activity in cerebrospinal syphilis, etc. Correlation appears to exist between cerebral and spinal fluid penetration, and the electronegative (anionic) character of bismuth, which is consistent with the comparatively greater penetration of other anions than cations.

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Streptococcus Leucocidin and the Resistance of Clasmatocytes.

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Although long known (Ruediger[1]), the leucocyte destroying properties of virulent hemolytic streptococci have never been as fully investigated as they deserve. We agree with McLeod[2] that leucocidin formation is perhaps the most important factor in the virulence of this micro-organism. We have recently undertaken a more complete analysis of leucocidin which we believe is the first since that of Channon and McLeod.[3] The only other study that is at all complete is that of Nakayama.[4]

The presence of leucocidin in a broth culture of streptococcus is evidenced by the demonstrable disintegration of leucocytes that are exposed to it, or better, by interference with the oxygen absorption of these cells when living as contrasted by its absence when they are dead. This change is delicately measured by the methylene blue bioscopic test of Neisser and Wechsberg.

The precise mode of action of leucocidin under conditions of in-

[1] Ruediger, *J. Am. Med. Assn.*, 1905, **44**, 198.

[2] McLeod, *J. Path. and Bact.*, 1914, **19**, 393.

[3] Channon and McLeod, *J. Path. and Bact.*, 1929, **32**, 283.

[4] Nakayama, *J. Inf. Dis.*, 1920, **27**, 270.

fection has not been made clear. In our opinion it is operative primarily within the leucocytes that have already ingested streptococci and for this and other reasons is separable from the negatively chemotactic substances known as "virulins" and aggressins. Nakayama believed the leucocidin separate from the hemotoxin also liberally produced by the streptococcus but Channon and McLeod question this. In our experiments leucocidin seems clearly separable from hemotoxin. In the first place leucocidin is not present in many hemolytic or hemotoxin-forming streptococci. Leucocidin continues to increase in inoculated broth for at least 48 hours and remains there for several days, whereas hemotoxin is present in greatest potency in from 8 to 12 hours and may entirely disappear by the end of 24 hours. Leucocidin is much more thermostable than hemotoxin; it resists heating to 65° for an hour, whereas 56°C. for one-half hour destroys hemotoxin. Leucocidin may actually be concentrated by evaporation at a moderate temperature. Leucocidin may be filtered through porcelain candles, which procedure diminishes or eliminates hemotoxin. Nakayama found the two substances separable by specific absorption.

Our streptococcus studies extending over many years have continued to prove more and more conclusively that tissue macrophages are superior to the polymorphonuclear leucocytes in various conditions of resistance and immunity and particularly in streptococcus infections. Impressed with the direct relationship of streptococcus leucocidin to streptococcus virulence, it is now of great interest to us to find that macrophages are much more resistant to leucocidin than are polymorphonuclear cells. A 3-day pleural exudate of the rabbit, containing about 50% clasmatocytes, reduces methylene blue 2 to 4 times as well as a 24-hour exudate which is largely polymorphonuclear. A given number of clasmatocytes, moreover, resists destruction by leucocidin in a dosage that completely abolishes respiratory activity in an aliquot portion of polymorphonuclears. In other words, the relatively perfect resistance of macrophages against leucocidin would seem to account for their superiority in defending the rabbit against virulent streptococcus infection.

5565

The Permeability of the Lymphatic Wall.

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The permeability of the walls of lymph channels can be studied with the aid of innocuous vital dye solutions isotonic with blood. When such solutions are injected with a micro pipette into the tissue near the edge of the upper surface of the ear of the mouse, the dye rapidly finds its way into the lymphatics draining the region, rendering them clearly visible. When one of the vessels carrying the colored fluid empties this latter into a larger channel draining a normal part of the ear, one sees the color swept away and diluted by a stream that is itself unseen. Lymph formation and flow are thus shown to be active processes in the ear, even in the absence of hyperemia.

Poorly diffusible dyes (Pontamine blue,[1] Chicago blue 6B, vital red, and Congo red), which pass with difficulty out of the blood vessels into the tissues, tend to be retained by the lymphatic wall as well, whereas more highly diffusible ones (trypan red, brom phenol blue) pass out with ease.

According to the accepted view, lymphatics are everywhere separated from tissue spaces by a continuous endothelial wall. Our observations support this view. When lymph carrying the dye that is retained by the lymphatic walls is put under pressure, it passes out into the tissues only when frank ecchymosis of it takes place. No sign is to be found of pre-existing lacunae in the wall.

Lymph flow is stopped by a pressure of 2 to 4 cm. of water exerted by way of a sausage-shaped, collodion bag laid across the lymphatics. The pressure obstacle causes the dye to escape in greater abundance than ordinarily. It does not interfere in the least with the venous channels but so effectively blocks the lymphatics that mild edema develops within the hour. The phenomenon is further proof that lymph formation is an active process in the ear.

Isotonic sodium chloride solution appears to exert an injurious effect upon the lymphatic endothelium, to judge from the rate of escape of dye dissolved in it. Tyrode's solution is much more tolerable as judged by this criterion, and so too with amniotic fluid of the mouse. Dye introduced in homologous serum diluted with 3

[1] Rous, Peyton, and Smith, F., *J. Exp. Med.*, 1931, **58**, 219.

parts of Tyrode solution to reduce the protein percentage approximately to that of lymph from an extremity escapes relatively slowly. When in undiluted serum, it is long retained, as would follow from the influence of the serum proteins.

5566

The Breakdown of Lymph Transport.

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The method described in a preceding paper to render visible the lymphatics coursing through normal tissue has provided us with the opportunity to study the permeability of the lymphatic wall under pathological conditions. Lymphatic permeability is greatly altered by slight causes, as can be shown by following the escape into the tissue of vital dyes ordinarily retained within the lymph channels.

When Pontamine blue or Chicago blue 6B, dyes having the requisite character, are introduced into the lymph, one sees the lymphatics of the ear as dark blue channels much broader in general than the blood vessels, and having the alternation of pear-shaped dilatations and constrictions seen in specimens injected after death. Their outlines are sharp because the dye does not pass from them into the tissue that they traverse. But a mere gentle stroke across their course, with a blunt instrument, at this time or just prior to the dye injection, results in an immediate escape of color into the region directly affected. This escape is closely localized to the line of the stroke and it endures for some minutes.

Greater degrees of disturbance of the lymphatics have proportional results. When unfiltered light from an arc is allowed to fall for a fraction of a minute upon a small area of the ear, there results an immediate and abundant escape into the tissue of the colored lymph. A similar phenomenon is to be seen when local inflammation has been produced with xylol.

These observations prove that the lymphatic wall becomes more permeable upon relatively slight stimulus, letting substances through into the tissues, which ordinarily it would retain. That the small blood vessels do this upon occasion is well known; and the process is held to be mainly responsible for urticaria, especially factitious urticaria. Incompetence of the lymphatics of urticarial regions may

very well be one cause for the accumulation of fluid in them. The wall of the lymphatics of regions that are inflamed may become so permeable as to fail largely, if not entirely, in the function of drainage.

5567

A Non-Metal Cage for Small Animals.

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The increasing use of small laboratory animals, especially the albino rat, in studies involving precise measurements has emphasized the importance of the care required in the technic of caging and feeding. Largely in response to the demands imposed by the recent investigations of nutritional anemia, several suggestions have been made for the design of cages in which the experimental animal has no access to metal. In connection with metabolism studies with a dietary regime extremely poor in inorganic salts, the same need for a metal-free cage arose in this laboratory and, because of the difficulty in cleaning the cages of the designs already suggested, the one herein described was devised. It has the advantages of being simple in construction, easy to wash and sterilize and consisting of parts which are standard and easily replaced (see accompanying illustrations).

The main portion of the cage consists of a Pyrex cylinder 21 cm. inside diameter, 22 cm. outside diameter and 16.5 cm. high. This rests on a grid made of a circular collar of galvanized iron 22.8 cm. in diameter and 5 cm. high, across which are bars consisting of glass tubing 3 mm. outside diameter. Copper wire No. 20 is run through the tubing for support and added strength and passes through holes in the collar at a distance of 1.5 cm. below the upper edge. There is a space of 0.7 cm. between the tubes forming the grid; this, however, can easily be varied to suit experimental conditions. The tubes are slightly bent so that urine dropping upon them tends to flow towards the center. A similar grid forms the top of the cage. If care is taken to make the tubes touch the metal collar, the animal has no opportunity to lick or chew metal.

The glass cylinder with the grids above and below sets in a heat-resistant pie plate, "Save all pie plate, Glassbake 600". In this plate

FIG. 1.

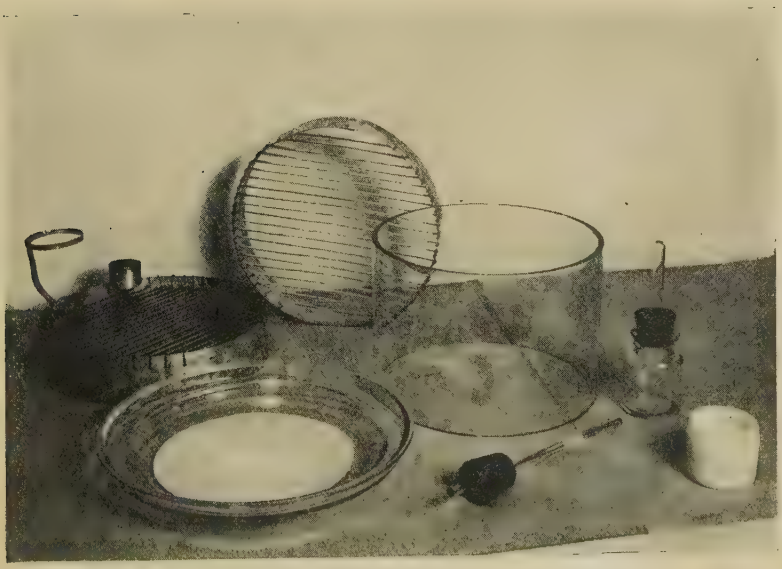

FIG. 2.

there is a shelf or flange half way up the bevelled edge; the cage rests on this shelf. The upper surface of the grid supporting the animal is 5.5 cm. above the bottom of the plate.

Food is provided in a glass cup kept in place by a glass rod, the upper end of which passes through a cork held in a ring on the upper grid collar. A water fountain of simple design is also supported from the collar of the upper grid. Urine and feces are collected on a double layer of acid-soaked filter paper. Satisfactory recovery of nitrogen, calcium and phosphorus has been obtained when the papers are changed at 3-day intervals. This cage prevents access to metals, eliminates coprophagy, consists of easily replaceable parts with relatively low cost and is readily cleaned and sterilized.

5568

Effect of Digitalis on Duration of Electrical Systole ("Q-T" Interval) in Cardiac Failure.

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Previous studies have shown that the "Q-T" interval of the human electrocardiogram is related to the duration of the cardiac cycle ("R-R" interval)[1] and that the "Q-T" interval is lengthened in relation to the cardiac cycle in cases of heart failure.[2] Since digitalis is the drug most successfully used in combating cardiac insufficiency, a study of the effect of this drug was made. It was found that adequate doses of digitalis almost uniformly produce a relative shortening of the "Q-T" interval and that a change in the same direction occurs in normal individuals[2] as well as in cardiac patients. Moreover, this effect of digitalis is seen at least as early as any other known change produced by its administration. A discussion with further details will be given in the complete paper.

The accompanying table gives a few examples of this effect of digitalis. Several hundred records, including those of 5 normal individuals, have been studied. In the table "K" is a constant in the

[1] Cheer, S. N., and Li, R. C., *Chin. J. Physiol.*, 1930, **4**, 191.

[2] Cheer, S. N., to be published.

TABLE I.
Effect of digitalis on "Q-T" interval.

Diagnosis	Date	"R-R" interval	"Q-T" interval	"K"	"T" lead II	Digitalis
Case 2		sec.	sec.		mm.	
Rheumatic mitral disease	June 6	0.580	0.330	0.433	9.0	None
	" 16	0.610	0.260	0.333	6.0	3.3 gm. in 10 days
Moderate heart failure	" 29	0.690	0.280	0.337	4.0	4.7 " " 23 "
	July 12	0.590	0.290	0.377	4.0	6.0 " " 36 "
Case 3						
Same, but severe failure	June 12	0.582	0.355	0.465	5.0	None
	" 14	0.660	0.330	0.406	4.0	1.4 gm. in 2 days
	" 19	0.580	0.270	0.355	4.0	2.2 " " 7 "
	" 28	0.568	0.275	0.365	4.0	2.7 " " 10 "
	July 10	0.590	0.270	0.352	4.0	3.3 " " 28 "
Case 5						
General arterio-sclerosis	Mar. 18	0.510	0.300	0.420	4.0	None
	" 19	0.550	0.315	0.425	4.0	1.2 gm. in 2 days
Hypertension	" 20	0.560	0.305	0.408	4.0	1.2 " " 3 "
Chr. nephritis	" 21	0.570	0.295	0.390	4.0	1.4 " " 4 "
Coronary thrombosis	" 24	0.597	0.285	0.369	4.0	2.0 " " 7 "

formula, "Q-T" interval $= \sqrt{K \text{ "R-R" interval}}$, so that "K" is an index of the ratio, "Q-T" interval : cycle length.

5569

Biochemical Studies of Human Semen. III. Factors Affecting Migration of Sperm Through the Cervix.*

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The viscous mucus which normally fills the canal of the cervix uteri presents a first barrier to the migration of spermatozoa from the vaginal lumen to the upper parts of the tract where fertilization occurs. When a mass of this mucus is exposed to the action of normal seminal fluid, the gross appearance is that of a lysis, with loss of viscosity and disintegration of the mass, apparently due to specific enzymic action.[1] This action is inhibited by the presence in the mucus of notable amounts of pus, leucorrheal cells, etc.

* This research was aided by a fund from The Chemical Foundation.

1 Kurzrok, R., and Miller, E. G., Jr., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 670; *Am. J. Obs. and Gyn.*, 1928, **15**, 56.

When the contact-boundary between normal cervical mucus and a normal semen specimen containing motile sperm is examined under the microscope, there is seen a gathering of sperm at the mucus surface which seems to be greater than can be accounted for merely by random swimming of the sperm. There is no evidence, however, of any attraction-field of the mucus surface exerting any influence on the distribution of sperm in the seminal fluid except in a narrow zone along the contact of the fluid with the mucus. The sperm do not very readily enter the mucus mass, nor do they progress in it, once entered, as rapidly as they do when swimming in a perfectly fluid medium. When one or more have succeeded in penetrating the mucus, what appears to be a small halo of more fluid material in the mucus can frequently be seen about the head of the advancing sperm, suggesting "lysis" of the mucus mass, thus making possible the advance of the sperm. Frequently, when one or more have entered, others follow, like a phalanx of sperm, with very actively lashing tails, following the leaders, oriented in general in the same direction, appearing to move up a "channel" against a vigorous current formed by the swimming-motion of the tails of those ahead; at times several sperm are swept backward and out by this current. It might possibly be suggested that the gathering of the many sperm in the column, oriented up-stream, might be due to a "rheotropism".

This picture is most noticeable immediately under the cover-slip, where the confinement by the glass tends to exaggerate the crowding, but it appears to be a phenomenon occurring generally at various places on the semen-mucus contact.

The "pioneers" of the sperm in this penetration tend finally to slow up, and become non-motile or very feebly moving, lying embedded in the viscous mucus, with random orientation. On exploring further into the mucus, away from the semen contact, occasional non-motile sperm may be seen. Only rarely can "free-swimming" cells be found except where the "mass penetration" by the phalanx of sperm has advanced.

The mucus plug in the cervix, then, seems to present an obstacle to the advance of the sperm; but it can be penetrated, and this penetration does not seem to be a mere random phenomenon depending only on the fortuitous direction of swim of the sperm in the seminal fluid.

As a possible factor in the first orientation of the sperm migration in the tract, we considered the occurrence of a potential gradient. The pH of the vaginal wall is usually about 3.6; of the

semen 7.6; the pH of the mucus plug may be as high as 8.5 or more. We should expect a potential difference to occur across the interphase betweeen semen and mucus, due to the great motility of H and OH ions, and to other factors. Semen and a mucus plug were brought in contact in a narrow tube; KCl-agar bridges connected either side of the contact with opposed calomel cells, which in turn were connected with a potentiometer-galvanometer set. The boundary potential so measured was of the order of 3 to 5 (or more) millivolts, the mucus being electropositive to the semen. The distance of the potential drop (the thickness of the phase boundary) may be estimated, at most, at 50 μ; this gives a potential gradient of about 1 volt per cm.

When mucus samples containing many pus cells were used for the microscopic test, penetration did not occur. These mucus samples were acid. A few similar mucus plugs, tested with semen as above for the phase-boundary potential, gave negative or small reversed potentials. We have previously shown that such mucus plugs are not affected by the dissolving action of normal semen.

Under the influence of an imposed E.M.F the sperm, which carry a negative charge, are drawn toward the positive electrode. The effect seems to be a cataphoresis rather than a galvanotropism, since it is not due to orientation of the swimming sperm cell toward the electrode. With low voltage gradients the cataphoretic effect is difficult to observe under the microscope, being largely masked by the active random swimming of the sperm. By placing semen in the middle chamber of a cataphoresis tube, layering Ringer solution or buffers on both sides, imposing a potential difference on the two ends, and then counting the sperm in the electrode chambers of the tube, we have observed a marked tendency of sperm to move into the positive field rather than into the negative, even with gradients as small as 0.01 volt per cm.